Food Research

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THE FREE AMINO ACIDS OF MUSTS AND WINES. III. EFFECT OF ADDED AMMONIA AND OF FERMENTATION TEMPERATURE ON THE FATE OF AMINO ACIDS DURING FERMENTATION

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Department of Viticulture and Enology, University of California, Davis

(Manuscript received June 9, 1958)

The amounts of seventeen amino acids in grape musts and their fate during the conversion of must to wine were reported in earlier papers *(2, 3, 4)*. Gerasimov, Saenko, and Chalenko *(5)* in their studies on the effect of the addition of phosphate and nitrogen on the fermentation of Crimean musts found that the addition of phosphate did not influence the rate of fermentation, but the addition of ammonium salts hastened it. Thorne *(10)* indicated that of 18 single amino acids presented to yeast in a defined nutrient medium, only one, aspartic acid, notably surpassed ammonium phosphate as a source of nitrogen for yeast culture growth. In a nutrient medium containing a complex mixture of amino acids, such as beer wort, non-ammonia nitrogen was taken up by the yeast more rapidly than ammonia nitrogen. The influence of added ammonia on yeast removal of amino acids from natural media, the possible related effects on yeast multiplication, ethanol production, and the residual level of amino acids in the fermented product were not completely defined. The effect of temperature was not determined in Thorne's studies.

This report presents the data of experiments in which the effect of added ammonia on the fate of 14 amino acids in fermenting must was studied at two fermentation temperatures. In addition, the changes in both naturally present and added ammonia nitrogen content, and in organic nitrogen (total nitrogen minus ammonia nitrogen) in relation to yeast cell multiplication and ethanol production were observed.

Experimental Procedure

General methods and materials. The variety of grape and strain of yeast employed were identical with those previously reported *(4)*. One hundred gallons of liquid must, pressed from freshly harvested and crushed French Colombard grapes, were placed in a stainless steel tank and mixed. The reducing sugar of the must was 22.4% and the pH was 3.60. Fifteen gallons of the must were distributed to each of 6 twenty-gallon glazed earthenware jars. The jars were arranged in 2 series, one for fermentation at 22° C and the other at 12° C. In each series, batch A was held as a control. The remaining 2 batches received additions of different ammonium salts nearly equivalent in nitrogen content. Batch B received 5.4 g. per gallon of diammonium phosphate ($(NH_4)_2HPO_4$); C received 4.7 g. of ammonium carbonate ($(NH_4)_2CO_3$). Each batch was heavily inoculated with a pure culture of the wine yeast (*Saccharomyces cerevisiae* variety *ellipsoideus* "Montrachet strain"). The initial yeast count in the several batches averaged 4.7×10^8 cells per 100 ml. The initial temperature of the must was 20° C. Series I was held in a room thermostatically-controlled at 22° C. Twelve hours after inoculation the batches of Series II were removed to a room thermostatically-controlled at 12° C and held there until fermentation was completed.

Sampling and analytical methods. Initial samples were taken immediately after the batches were inoculated and stirred. Thereafter, samples were collected at predetermined

[a] Prepared for publication by T. E. Archer.

intervals. Sampling, yeast cell counts, alcohol analyses, and amino acid assays were carried out as in earlier reports *(3, 4)*. Total nitrogen was determined by the Kjeldahl method for wine *(1)* with copper shot used as a digestion catalyst. Ammonia nitrogen was determined by steam distillation of samples made alkaline with saturated potassium carbonate. The ammonia was trapped in standardized hydrochloric acid, and the acid was back titrated while hot with standardized sodium hydroxide. A vacuum jacketed micro-Kjeldahl distilling flask was used *(9)*. Experiments with ammonia nitrogen added to samples indicated recoveries of 96 to 98%.

RESULTS AND DISCUSSION

Only the averages of the composite data obtained from the batches of Series I and Series II are presented in Figures 1 to 4. This was done for convenience in plotting and reading the graphs. Only plainly significant variations from the control patterns were plotted. The results, shown in Figures 1 to 4, indicate that at the higher fermentation temperature (22° C.), added ammonia nitrogen produced marked stimulation of yeast multiplication and ethanol production with relatively little effect on the rate and amount of removal of most of the amino acids investigated. At the lower fermentation temperature (12° C) no stimulating effect of added ammonia on yeast multiplication and ethanol production was observed. The fermentation temperature of 22° C. was selected since it is a medium range temperature for fermentation, and many California wines are fermented at this temperature. The fermentation temperature of 12° C. was selected because the yeast employed ("Montrachet strain") can ferment well at this temperature yet yeast cell autolysis might be minimized at this low temperature. Under conditions where yeast cell multiplication is prevented but enzyme activity is possible, as in the latter stages of fermentation, cellular autolysis can presumably occur. Autolysis could account for the return of several amino acids to the fermenting must. Deviations from the normal pattern of the utilization of the amino acids, as reflected by the control fermentations, appeared as variations in the return of the amino acids to the fermenting medium after the initial rapid yeast cell multiplication.

Effect of added ammonia on fermentation processes. The effect of added ammonia on yeast cell multiplication, ethanol production and temperature changes during fermentation are shown in Figure 1. The same two overlapping phases of fermentation (Phase I, formation of yeast cells and their enzyme systems; Phase II major formation of fermentation products) found in earlier work *(4)*, and previously pointed out by Lee *(8)* for industrial fermentations in general, appeared in the present work. Added ammonia, at the higher fermentation temperature, somewhat lengthened Phase I, and increased the yeast cell count in comparison with the control. At the lower fermentation temperature the stimulating effect of added ammonia on the rate and amount of the yeast cell multiplication was absent. The maximum yeast cell count and the duration of Phase I were practically identical with and without added ammonia. At 22° C. Phase II was affected by added ammonia. With added ammonia, ethanol formation was completed 24 hours earlier than in the control (Figure 1). At 12° C. fermentations were slower, and addition of ammonia gave no significant variation from the control. The final amounts of ethanol produced in all the fermentations were practically identical at both temperatures. It appears that at the higher temperature, the earlier completion of Phase II with added ammonia can be attributed to the formation of larger

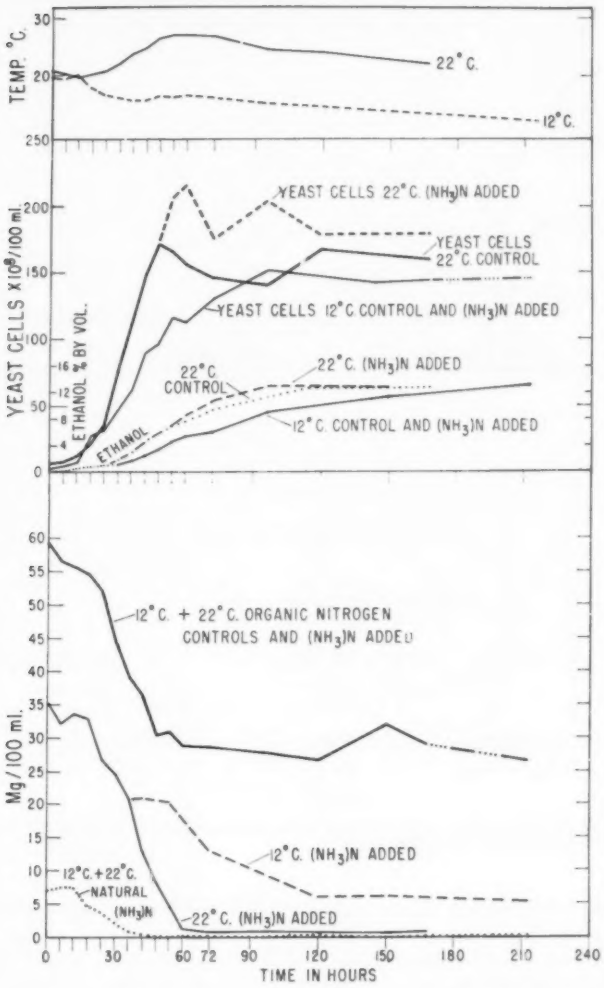

Figure 1. The effect of temperature and added nitrogen on yeast multiplication, alcohol production, and changes in total and ammonia nitrogen during fermentation of must to wine.

amounts of active enzyme systems (higher yeast cell count). The role of temperature in modifying the pattern of yeast cell multiplication and ethanol formation, probably through its effect on enzyme activity, was also clearly indicated.

Effect of added ammonia on amino acid changes. Most of the amino acids considered showed a large decrease in amount during Phase I of the

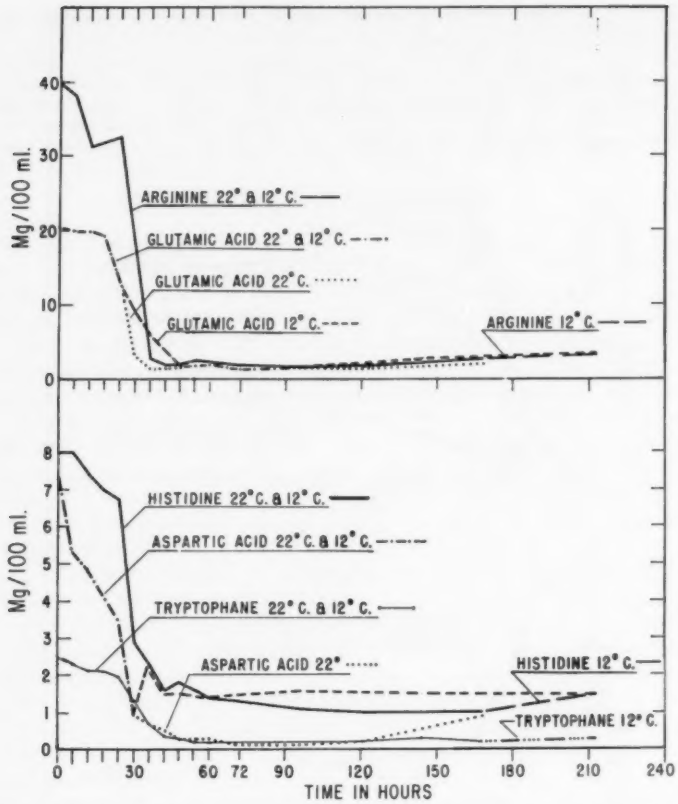

Figure 2. Effect of temperature and of added nitrogen on changes in amounts of amino acids during fermentation.

fermentations (Figures 2, 3, 4). The pattern of decrease was generally very similar to that found in earlier work *(4)*. Eleven of the amino acids—arginine, glutamic acid, valine, isoleucine, leucine, histidine, aspartic acid, tryptophane, tyrosine, phenylalanine, and methionine—were largely removed from the must by the yeast. Three amino acids, glycine, lysine, and cystine, present in the must in small amounts, showed little or no change in amount during fermentation. The rapid decrease of amino acids in Phase I, with concurrent rapid increase in yeast cell number, presumably represents the absorption and conversion of amino nitrogen to yeast protein.

With respect to the effect of added ammonia upon the degree of utilization, 11 amino acids—arginine, glycine, lysine, cystine, isoleucine, histidine, methionine, phenylalanine, tryptophane, tyrosine, and valine—showed no significant variations from the pattern established by the controls. The pattern of decrease was also very similar at the two temperatures and did not noticeably

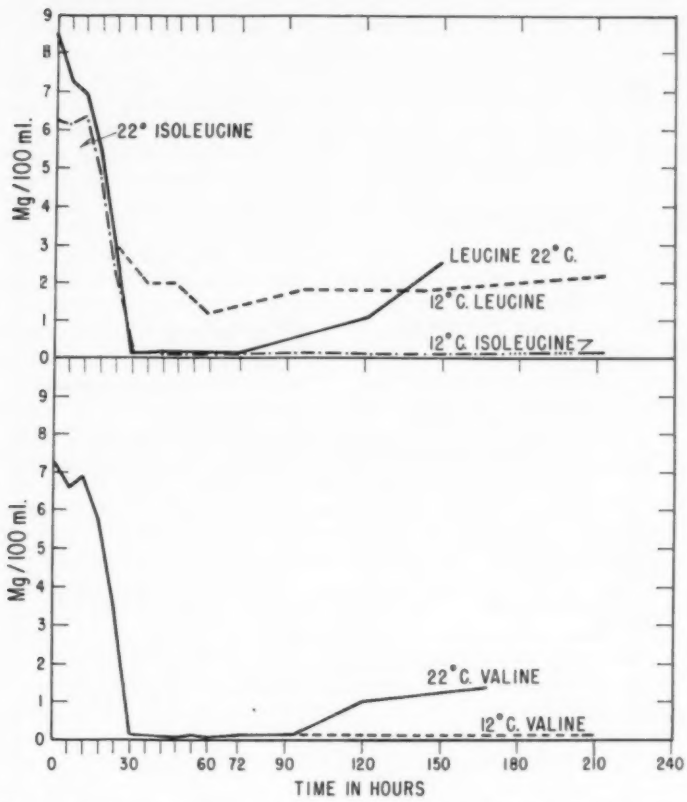

Figure 3. Effect of temperature and of added nitrogen on change in amounts of amino acids during fermentation.

reflect the slower yeast cell multiplication and smaller final cell count of the fermentations at 12° C.

Changes in the amounts of glutamic and aspartic acids (Figure 2), and leucine (Figure 3), showed variations from the control pattern. Variations were apparently related to the differences in the fermentation temperatures and not to added ammonia. The rapid decrease of aspartic and glutamic acids at the higher temperature, even in the presence of added ammonia, coincides with Thorne's observation *(10)* that singly they are somewhat better than ammonia nitrogen as sources of nitrogen for yeast culture growth. Although leucine ranks high as a source of nitrogen for yeast growth, it does not rank as high as ammonia nitrogen. For all the amino acids, except glycine, lysine, and cystine, the larger decrease in amount during Phase I ceased 10 to 35 hours before rapid yeast cell multiplication (Phase I) ended.

In Phase II, the pattern of change of amino acids showed variations chiefly in the amount returned to the media in the latter stages of fermentation.

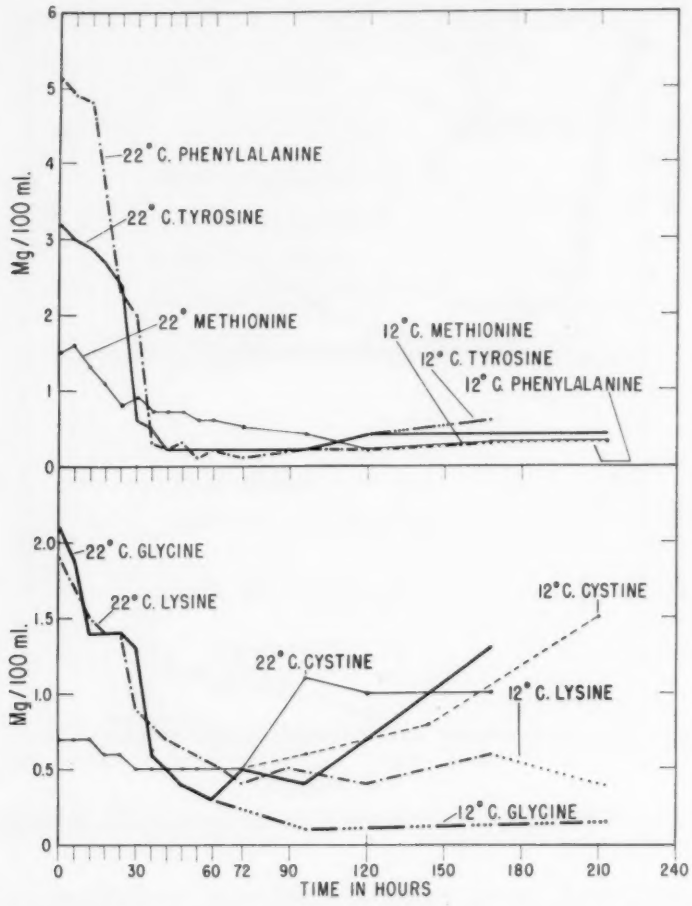

Figure 4. Effect of temperature and of added nitrogen on changes in amounts of amino acids during fermentation.

Variations appeared in the return of glycine, cystine, leucine, valine, and glutamic and aspartic acids. The pattern of change for glycine (Figure 4) indicated that at the higher fementation temperature return to the media was greater in the presence of added ammonia than in the control. At the lower temperature, the final glycine level was the same in the control and with added ammonia. At both temperatures, the pattern of change for cystine (Figure 4), indicated that the return to the media was higher in the presence of ammonia than in the controls. The return of leucine (Figure 3) to the media in Phase II seemed to be unaffected by the presence of ammonia. Valine (Figure 3) return to the media was unaffected by the presence of ammonia but was greater at the higher temperature. With glutamic and

aspartic acids (Figure 2), ammonia nitrogen appeared to cause no significant variation from the control at either temperature. Two of the 8 remaining amino acids, tyrosine and phenylalanine (Figure 4), showed a definite return to the media with no variation from the control as to effect of added nitrogen. The effect of ammonia nitrogen on the return of the amino acids to the media in Phase II of the fermentations shown here was in general agreement with the results of earlier unreported experiments in which the effect of added ammonium phosphate alone was studied.

Effect of temperature on amino acid changes. The effect of temperature upon the fate of the amino acids was more apparent than the effect of added ammonia nitrogen. Arginine, cystine, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, trytophane, tyrosine, and valine (Figures 1 to 4) utilization in Phase I was unaffected by both temperature and added ammonia nitrogen. In Phase II at 22° C. there was a high return of glycine (Figure 4) to the media and no return at 12° C. The return of cystine (Figure 4) to the media in Phase II at 12° C. was higher than at 22° C. At 22° C. there was an appreciable return of valine (Figure 3) to the media in Phase II and no return at 12° C. There was only a slight return of histidine (Figure 2) to the media in Phase II at 12° C. The other 7 amino acids of this group had no return to the media in Phase II at either temperature.

Glutamic acid (Figure 2) decreased more rapidly at 22° C. in Phase I, and in Phase II slightly more amino acid was returned to the media at the lower temperature than at the higher temperature. Aspartic acid (Figure 2) was reduced more rapidly and to a lower amount in Phase I at 22° C., but some return was indicated in Phase II. At 12° C. less aspartic acid was removed in Phase I and less was returned in Phase II of the fermentations. In Phase I leucine (Figure 3) decreased in all the fermentations more rapidly and to a lower level at the higher fermentation temperature. In Phase II there was more return of leucine to the media at 22° C. than at 12° C. In general those amino acids that were affected by the temperature differences reflected a more rapid utilization in Phase I and greater return to the media at 22° C. than at 12° C.

Fate of ammonia and organic nitrogen. Although the effect of added ammonia on the pattern of decrease of many amino acids was relatively small, it caused a marked increase in yeast cell count. It was considered of interest to determine the changes in ammonia nitrogen and non-ammonia total nitrogen. These changes are shown in Figure 1. The ammonia nitrogen naturally present in the must was approximately 11% of the total nitrogen. During Phase I of the control fermentations at both temperatures, naturally-present ammonia disappeared from the fermenting must within 48 hours after the fermentations were started. No ammonia was returned during Phase II of the fermentations. This observation agrees with the observation of Thorne *(10)* that although the total nitrogen may increase in amount during the later stages of fermentation of beer wort, ammonia nitrogen is not included. Added ammonia nitrogen, at the higher fermentation temperature, practically vanished at the 60th hour after fermentation was started. Only traces of ammonia were detectable during Phase II. At the lower fermentation temperature, added ammonia slowly decreased during Phase I and the early part of Phase II to a level of 5 to 6 mg. per 100 ml. by 144 hours, and remained practically constant thereafter.

The total nitrogen of wines is considered to consist of ammonia, albuminoid, phosphomolybdate precipitable, amino, amide, and humin nitrogen with an unidentified residual fraction amounting to 26% of the total *(7)*. A review of the chemistry of musts and wines mentions no nitrites and only traces of nitrates *(6)*. It appears reasonable to assume that the non-ammonia nitrogen of musts and wines is organic nitrogen. Organic nitrogen was determined in fermentation samples as the difference between ammonia nitrogen and total nitrogen. The organic nitrogen of all fermentations decreased at nearly the same rate until the 48th hour after inoculation. Thereafter, the level of organic nitrogen remained almost constant throughout the remainder of the fermentation period. The mean final loss of organic nitrogen was 48% of the initial value in the must. Although some gains and losses of amino acids in the fermenting must after the 48th hour (Figures 2, 3, and 4) occurred, calculations indicated that the changes were too small to be definitely reflected in the organic nitrogen data of Phase II.

SUMMARY AND CONCLUSIONS

The effect of added ammonia nitrogen and of fermentation temperature on the fate of fourteen amino acids was followed during fermentation of must to wine. At 22° C. added ammonia nitrogen increased the amount of the yeast multiplication and increased the rate of ethanol production. At 12° C. addition of ammonia nitrogen had no effect on yeast multiplication and ethanol production.

The pattern of amino acid utilization at both temperatures with added ammonia nitrogen showed no significant variations from that of the controls for eleven of the fourteen amino acids. Variations in the changes in the amounts of glutamic and aspartic acids and leucine from the control pattern were apparently related to the differences in the fermentation temperatures and not to added ammonia nitrogen. Deviations from the normal pattern of the utilization of the amino acids at both fermentation temperatures appeared during the period of formation of the major portion of the fermentation products (Phase II). In Phase II, the pattern of change of the amino acids showed variations chiefly in the amounts returned to the media in the latter stages of fermentation. The effect of temperature and added ammonia nitrogen on the return of the different amino acids to the media was inconsistent.

The ammonia nitrogen present in the must amounted to 11% of the total nitrogen and disappeared 48 hours after the fermentations were inoculated. No ammonia was returned to the media in Phase II. Added ammonia nitrogen in the 22° C. fermentations disappeared by the 60th hour of fermentation and only traces were detected in Phase II. At 12° C. added ammonia nitrogen slowly decreased to 5 to 6 mg. per 100 ml. by 144 hours and decreased no more thereafter.

The organic nitrogen of all fermentations decreased in amount at the same rate until the 48th hour after inoculation and thereafter remained nearly constant during the remainder of the fermentation period. The mean final loss of organic nitrogen was 48% of the initial amount in the must.

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CHANGES IN THE MALIC AND TARTARIC ACID CONTENTS OF CONCORD GRAPES[a]

W. B. ROBINSON, NELSON SHAULIS, GEORGE C. SMITH[b] AND DONALD F. TALLMAN
New York State Agricultural Experiment Station, Geneva, New York

(Manuscript received July 25, 1958)

In a previous study of factors affecting the composition of Concord grape juice *(11)*, the sugar-acid ratio was found to be affected most by two factors, season and pruning severity. Trellis height exerted a lesser influence. When maturity was defined in terms of soluble solids accumulation, heat units were not particularly useful as a measure of maturity. Indications that acid contents were less affected than sugar contents by cultural practice variations encouraged the present investigation into the changes in acidic constituents of Concord grape juice during ripening and from season to season. The acids of grapes have been the object of much research since the nineteenth century *(3)*, but the causes of their variation are still subject to much conjecture *(2, 8)*.

EXPERIMENTAL

The grapes used in this study were obtained from vines of the Concord variety grown in New York State. Vine training, pruning, and sampling procedures and juice preparation have been described in a previous publication *(11)*.

Total acid was determined by titration to pH 8.0 and is expressed as tartaric acid. Tartaric acid was determined by the metavanadate method *(5)*. Malic acid was determined by the method of Goodban and Stark *(4)*.

Heat units for each day were determined by subtracting 50 from the average of the maximum and minimum temperatures in degrees Fahrenheit. The daily units were accumulated from date of bloom to date of harvest.

RESULTS

In 1956 comparisons were made (Table 1) of the sugar, acid, and tartrate changes in Concord grapes as affected by very severe, medium, and very light prunings *(10)*. Ten, 40, and 70 buds, respectively, were left on the vine for the initial pound of prunings and 10 buds for each additional pound. Pruning exerted a pronounced effect on sugar content (Fig. 1), but had little, if any, effect on the acidity of the grape. As the grapes ripened a change occurred in the character of the acidic constituents. On September 6, when the grapes were still in the red stage, tartrates comprised less than 30% of the acids, but as ripening progressed, there was little change in the tartrate content while the total acids decreased gradually so that tartaric acid became the major fraction at harvest (Figure 2). Since the acids were unaffected by pruning severity differences, the sugar-acid ratio values follow the same pattern as that of the soluble solids readings.

In view of the relative stability of the acids of grapes to changes in pruning severity, it was thought probable that the acids would be less influenced than sugars by factors affecting the leaf-to-fruit ratio such as leaf exposure, crop load, and cluster thinning. With this in mind, it was decided to reassess the value of heat units from the standpoint of acid content of the grapes. Samples of grape juice were available for eight different years. For each of these samples the dates of bloom and harvest and appropriate temperature data were available. These samples were analyzed for malic acid content, total acid, and soluble solids (Table 2).

---

[a] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1138, July 18, 1958.

[b] Present address: Campbell Soup Company, Camden, New Jersey.

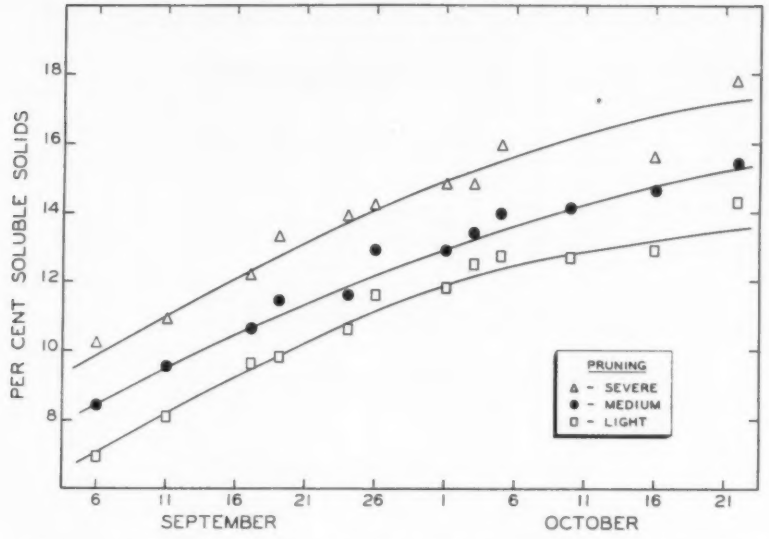

Figure 1. The development of soluble solids in ripening Concord grapes as affected by pruning severity.

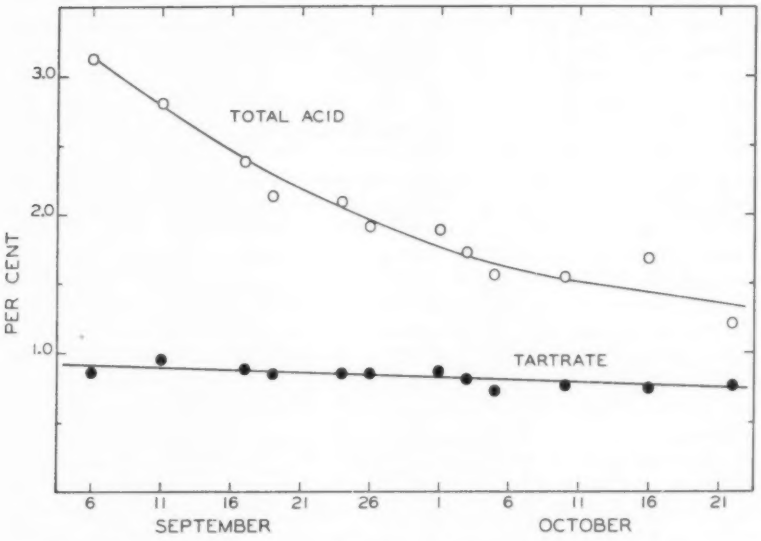

Figure 2. Changes in acid content of Concord grapes during ripening.

TABLE 1

Seasonal changes in the composition of Concord grapes at three cropping levels in 1956

Date 1956		Pruning Severity [1]												
		Heavy	Medium	Light	Heavy	Medium	Light	Ave.	Heavy	Medium	Light	Ave.	Heavy	Medium	Light
		Soluble Solids, %			*Total acid, as tartaric, %*				*Tartrate, %*				*Sugar-acid ratio*		
September	6	10.2	8.4	6.9	3.17	3.06	3.14	3.12	0.72	0.98	0.87	0.86	3.2	2.7	2.2
	11	10.9	9.5	8.1	2.66	2.71	3.06	2.81	0.92	0.99	0.98	0.96	4.1	3.5	2.6
	17	12.2	10.6	9.6	2.27	2.33	2.55	2.38	0.84	0.87	0.92	0.88	5.4	4.6	3.8
	19	13.3	11.4	9.8	1.85	2.20	2.35	2.13	0.79	0.85	0.92	0.85	7.2	5.2	4.2
	24	13.8	11.6	10.6	1.90	2.13	2.21	2.08	0.84	0.85	0.87	0.85	7.3	5.4	4.8
	26	14.2	12.9	11.6	1.85	1.94	1.95	1.91	0.84	0.86	0.85	0.85	7.7	6.6	6.0
October	1	14.8	12.9	11.8	1.78	1.94	1.95	1.89	0.91	0.84	0.84	0.86	8.3	6.6	6.1
	3	14.8	13.4	12.5	1.65	1.75	1.75	1.72	0.82	0.83	0.79	0.81	9.0	7.7	7.1
	5	15.9	14.0	12.7	1.52	1.55	1.62	1.56	0.68	0.75	0.73	0.72	10.4	9.0	7.8
	10	——	14.1	12.7	——	1.55	1.55	1.55	——	0.83	0.71	0.77	——	9.1	8.2
	16	15.6	14.6	12.9	1.63	1.69	1.65	1.68	0.74	0.74	0.78	0.75	9.6	8.6	7.8
	22	17.8	15.4	14.3	1.30	1.19	1.16	1.21	0.69	0.76	0.82	0.76	13.7	12.9	9.2

[1] The yield per acre for these three pruning treatments were 2, 8, and 14 tons, respectively (12).

TABLE 2

The relation of heat units to composition of Concord grapes in eight different years

Year	Malate	Total acid	Soluble solids	Harvest date	Heat units
	%	%	%		
1949	0.28	0.78	18.4	9/8	2127
1950	0.44	1.13	13.8	10/11	1819
1951	0.33	0.76	15.4	10/10	1952
1952	0.36	0.77	19.2	10/1	2099
1954	0.37	0.86	15.5	10/8	2086
1955	0.22	0.64	16.8	10/4	2409
1956	0.71	1.30	16.0	10/16	1790
1957	0.31	0.65	18.2	10/9	2030

The degree of correlation with heat units for the various constituents is shown by the following coefficients:

Variable	r value	significance
Soluble solids	+0.48	n.s
Total acid	−0.78	*
Malic acid	−0.78	*
Tartaric acid [1]	−0.61	n.s

* Significant at 5% level; n.s. — not significant.

[1] Total acid minus malic acid.

DISCUSSION

The New York State studies on Concord grapes reported here confirm the relationship between environmental temperatures and grape acid that has been observed in France *(6, 7)* and California *(1, 13)*. Peynaud and Maurie have reported recently that the ratio of malic to tartaric acid varied from year to year at comparative maturities, but not necessarily according to temperature variations *(8)*. Ribereau-Gayon had reported that temperature is only one factor in the relationship *(9)*. According to Amerine *(2)* the per cent malate and the per cent titratable acidity due to malates is greater, the cooler the grape-growing region. As a result of the present study, we may add "the cooler the season" to the Amerine rule. Perhaps the apparent discrepancies occasionally reported by the French investigators may be caused by defining maturity in terms of sugar content. The present authors *(11)*, using soluble solids as a criterion, reported that heat units were not particularly useful as a measure of maturity. From the seasonal changes in total acid and tartrate (Figure 2) and from the soluble solids data (Table 1), it can be deduced that at 14.2% soluble solids there was a higher content of malic acid on September 26 with severe pruning than on October 22 with light pruning. Winkler observed that as the crop of vinifera grapes increased the attainment of a given degree Balling was delayed. The delay in each instance was accompanied by a decrease in acidity *(13)*.

The data presented here indicate that malic acid is more responsive to heat units than is tartaric acid. This was indicated in 1956 when the predominant changes in the total acid as ripening progressed were caused by a decrease in the malic acid. The response of malic acid content to temperature in different seasons was shown by the significant negative correlation with heat units as opposed to insignificant correlation with heat units for the

remainder of the total acid which, of course, is predominantly tartaric (Table 2).

SUMMARY

Evidence is presented to show that the malic acid and total acid of Concord grapes is significantly related to the heat units accumulated between the dates of bloom and harvest. Soluble solids and tartaric acid were not significantly correlated with heat units. Pruning severity affected the soluble solids of the grapes but had no effect on the rate of acid-content decline. The possibility that leaf-to-fruit ratio or leaf exposure may exert more influence on soluble solids than on malic acid content may explain the fact that correlation with heat units is obtained in the case of malic acid, but not in the case of soluble solids.

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BROWNING OF SUGAR SOLUTIONS. 3. EFFECT OF pH ON THE COLOR PRODUCED IN DILUTE GLUCOSE SOLUTIONS CONTAINING AMINO ACIDS WITH THE AMINO GROUP IN DIFFERENT POSITIONS IN THE MOLECULE[a]

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In the first paper of this series *(11)* it was reported that when dilute glucose solutions containing various types of amino acids were heated at 114° C. for 20 minutes, the presence of lysine, a diaminomonocarboxylic acid, caused the formation of more color than did alanine, a monoaminomonocarboxylic acid or glutamic acid, a monoaminodicarboxylic acid. Also, at the dilute concentration of glucose and amino acids used, over a pH range of 5 to 9, only those solutions containing lysine developed significantly more color than a solution containing only glucose. Further, in none of the solutions except those containing lysine was color produced below pH 7. The presence of lysine resulted in some color at pH 6. Generally, more color was produced at higher pH values — an observation made by many authors *(1, 2, 3, 4, 6, 8, 9, 10)*.

The general basic nature of lysine does not explain its increased activity in color production, since histidine and arginine caused no increase in color over the glucose control. Therefore a second study *(7)* was made to determine the effect of the position of the amino group in the molecule on the production of color at pH 8 in dilute glucose-amino acid solutions. The compounds used were homologs of the 2, 3, 4, 5 and 6 carbon fatty acids containing amino groups (a) on the alpha carbon, (b) on the omega carbon, and (c) on both the alpha and the omega carbon atoms. The alkaline medium was used because definite color was produced by glucose alone at this pH and differences in color development would indicate the relative effect of the various amino acids. These amino acids influenced the amount of color produced to varying degrees *(7)*. Therefore, it was deemed important to determine whether these variations are influenced by the pH of the solution. This paper deals with the effect of pH on the influence of the position of the amino groups in amino acids in the production of color in dilute glucose solutions. The pH effect in the range 5 to 7 was studied because it has been noted in the previous papers that certain amino acids caused sugar solutions to brown slightly below pH 7. As in the earlier work, dilute concentrations of glucose and the amino acids were used in order that differences in pH effect might be readily seen.

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[a] Presented at the 133rd National Meeting of the American Chemical Society, San Francisco, California, April 13-18, 1958.

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EXPERIMENTAL

That amount of each 12 amino acids* was added to 0.1 molar glucose solutions to give 0.1 molar concentrations of the amino acids. Seven series of the 12 amino acid-glucose solutions were made and buffered with the appropriate phosphate buffer at pH 5, 6, 6.5, 6.8, 7, 8, and 9. Five milliliter aliquots of each of these solutions and of glucose controls were heated in sealed tubes at 114° C. as described previously (11). As earlier work had shown that the amino acids would not develop color under the conditions of the study, no amino acid controls were run in this experiment. After cooling, the absorbances of the solutions were measured in 1 cm. cells at 500 mμ in a Cary recording spectrophotometer. The data are presented in Table 1.

RESULTS AND DISCUSSION

The general effect of pH on the reaction between different amino compounds and sugars has been reported by several authors. Borsook and Wastenays (2) found that the reaction as followed by reduction of amino nitrogen was favored by an alkaline medium. Frankel and Katchalsky *(3, 4)* using titration measurements also reported that the α-amino acids and sugars react to a greater extent the higher the pH. The present study revealed the same trend, including those for solutions containing only glucose which had zero absorbance at pH 5, 6, and 6.5 and absorbances of 0.03, 0.08, 0.64 and 0.75 for pH's at 6.8, 7.0, 8.0 and 9.0, respectively. Also, the same variability reported previously *(7)* on the influence among different types of amino acids was again evident. The effect of pH on this variability is illustrated in Figure 1. Since the α-amino acids had little influence on the amount of color pro-

* The amino acids used in the study were glycine, DL-α-alanine, DL-β-alanine, DL-α-aminobutyric acid, DL-γ-aminobutyric acid, 2, 4-diamobutyric acid, DL-norvaline, Δ-aminovaleric acid, DL-ornithine • HCl, DL-norleucine, ψ-aminocaproic acid, and DL-lysine • HCl.

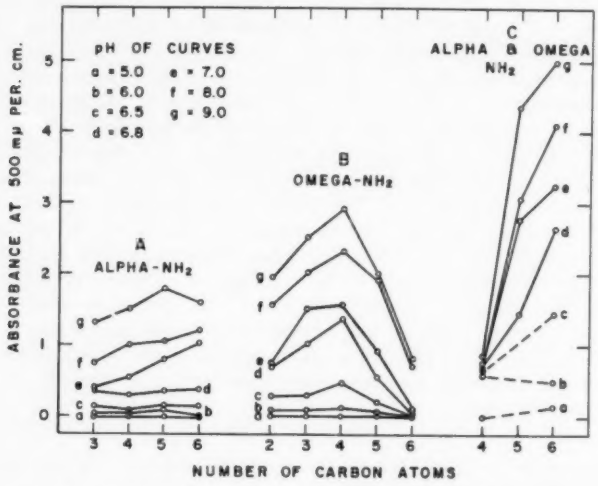

Figure 1. Effect of pH on vanability of reaction between amino compounds and mgars.

duced at pH 8, a change of pH was expected to produce only a small change in their influence. This is confirmed in Figure 1A. In the two series, containing the ω-monoamino and the α, ω-diamino acids, where the position of the amino group and chain length of the acids caused a significant difference in the amount of color produced, the effect of the amino group was accentuated by increasing the pH (Fig. 1B and 1C). In Figure 1B the color producing effect of the chain length of the amino acids containing only omega positioned amino groups is compared at different pH values. Here the amount of color produced at pH 5 and 6 by all the compounds was so small that differences due to chain length were negligible. At pH 6.8 an appreciable amount of color was produced and this differed from that developed at higher pH values only in amount. It should be noted, therefore, that the positive effect of these acids on color is not limited to pH values above 7, but under the conditions of this study continue down to at least pH 6.5. Further, the difference in the amount of color produced appears to be due to an accentuated effect of the amino group as the pH was raised.

Figure 1C shows that pH has an even greater influence on the browning effect of the diamino acids.

These data indicate that the amount of color produced in glucose-amino acid solutions is a function of (a) the pH, (b) the chain length of the acid, and (c) the number and position of the amino groups. The greatest effect of the pH was with solutions containing diamino acids and the least effect was with the α-amino acids. Even though the greatest effect on color was demonstrated by the diamino acids the pronounced increase with increased pH cannot be attributed only to the doubling of the number of amino groups. Were this true, the solutions of diamino acids of a given number of carbon atoms would have (a) browned only twice as much as the monoamino acid solution or (b) had a color value equal to the algebraic sum of the amounts of color formed by both the α-amino and the ω-amino acids of the equivalent chain lengths. Neither of these obtains.

CONCLUSION

This study shows the effect of pH on the amount of color formed in heated dilute glucose solutions containing a straight chain amino acid of three homologous series containing either an alpha or an omega amino group or both, and a chain length of two to six carbons. The pattern of the pH effect is different for the three homologous series but similar for the acids within each series.

In the α-amino acid series little color was produced, but there was a significant increase from pH 7 to 9.

In the ω-amino acid series there was no definite effect at pH 5 and 6, but from 6.5 color production increased progressively with increase of pH up to 9.0. The characteristic item of this series was the maximum effect at a chain length of four carbons.

In the α, ω-amino acid series little color was produced at any pH by the 4 carbon acids, but with the 5- and 6-carbon acids color was greatly increased, especially at the highest pH values. At pH 5 and 6 there was no increase in color with increase in chain length.

Considering the pH effect, especially in the critical range between 5 and 7, the following is observed in the three series of amino acid-glucose solutions:

(a) At pH 5 no browning occurred.

(b) At pH 6 only the α, ω-diamino acid series produced appreciable browning.

(c) At pH 6.5 the ω-amino acid of 4 carbons as well as the α, ω-acids produced browning.

(d) At pH 6.8 both the ω (up to 4 carbon atoms) and the α, ω-acids produced significant browning.

(e) At pH 7 and above all of the series produced browning.

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EFFECT OF STORAGE CONDITIONS ON SOME OF THE BIOCHEMICAL PROPERTIES OF MEAT AND ON THE PHYSICAL PROPERTIES OF AN EXPERIMENTAL SAUSAGE

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In the manufacture of sausage, the quality of the meat is one of the most important concerns confronting the processor. Sausage products made from unsuitable materials are usually of poor texture, chiefly because the water and fat of the meat have been released from the product. Such products are not acceptable, as a rule, on the commercial market.

Many articles *(1, 4, 6, 7, 8, 21, 22, 24, 25)* have been published on the quality of meat, most of them based on the water-holding capacity of the meat under study. It is suggested in many of these articles that actomyosin, one of the structural proteins of muscle, might play an important role in meat quality. The present authors *(26)* have recently reported on the mechanism of the denaturation of myosin B (natural actomyosin), indicating that the denaturation proceeds in two steps, viz., (a) a rapid first-order reaction which causes partial denaturation and (b) a pH dependent reaction that proceeds slowly to complete denaturation. This latter reaction is "superimposed" upon the former.

It is plausible to suppose that the denaturation of actomyosin may be closely related to the quality of the sausage, since actomyosin is the most important constituent of muscle protein. In line with this concept, experiments were initiated to determine whether or not the denaturation of myosin B in muscle during storage proceeds in a manner similar to that of isolated myosin B. A further objective was to determine whether the elasticity of sausage decreases in accordance with the denaturation of the protein.

EXPERIMENTAL

1. Materials

Rabbit skeletal muscles were the chief materials used. Beef *M. semitendineus* was also used in a special experiment.

Muscle homogenates were prepared by means of a glass-homogenizer; about 20 ml. of water was added to 10 g. of ground meat.

Myosin B was extracted from the muscle with the Weber-Edsall solution for 24 hours and purified by the usual dilution-precipitation method.

Adenosine triphosphate (ATP) was isolated from fresh rabbit muscle by the method of Kerr *(10)* and used as the potassium salt.

2. Procedure

Adenosine triphosphatase (ATPase) activity. Nine ml. of 0.6 M. potassium chloride solution of homogenate or myosin B containing 1/20 M. tris(hydroxymethyl)aminomethane-maleate buffer (pH 6.9) and 10^{-2}M. calcium chloride, 10^{-3}M. magnesium chloride or 10^{-2}M. ethylendiaminetetraacetate (EDTA), was kept in constant temperature bath at 20° C. for 5 minutes; 1 ml. of 10^{-2}M. ATP was added to the above mixture, thus starting the reaction.

At measured intervals of time aliquots of the reaction mixture were pipetted into equal volume of 10% perchloric acid. The protein precipitated was removed by filtration through a dry filter paper. The amount of phosphate liberated was determined colorimetrically, using the Fiske-Subbarow method.

Salting-out analysis was made, employing the technique of Derrien *(5)*. Experimental conditions were almost the same as those under which Snellmann and Tenow *(20)* carried on their work. Ammonium sulfate solution of various grades of saturation were prepared. To 5 ml. of this solution was added 1 ml. of protein solution (0.6 M. potassium chloride) and the mixture was allowed to stand at 1° C. for 20 hours. Then the precipitate was filtered off and the protein concentration of the filtrate was measured by Folin's phenol reagent *(16)* with careful attention to the inhibitory action of excess ammonium sulfate.

The nitrogen extractable with the Weber-Edsall solution was estimated in the following way. About 5 g. of sample meat was ground and weighed, then suspended in 50 ml. of cold Weber-Edsall solution and kept in refrigerator for 24 hours, being stirred occasionally. After this process, the suspension was centrifuged and the supernatant fluid was filtered through a cloth. The residue was washed several times with Weber-Edsall solution by centrifugation. The washing solution was combined with the first supernatant fluid in a measuring flask and the total volume was made up to 200 ml. An aliquot of this solution was pipetted into a digestion flask and nitrogen content was determined by the micro-Kjeldahl method. The same method was also used in the other types of nitrogen determination in this paper. The protein content was calculated by multiplying the nitrogen content by a factor 6.25.

The pH values were determined by the use of a glass electrode pH meter.

The pH value of meat was controlled by starvation of the animal for 48 hours or by injection of insulin before slaughter, according to the method of Bate-Smith and Bendall *(2)*.

The sample sausages were prepared according to commercial practice but with the addition of 2.5% sodium chloride (W/W) to the ground meat. Sausages were well mixed and stuffed into casings of gum hydrochloride (31 mm. in diameter) and cooked for 30 minutes in water-bath at 70° C.

The quality of the sausage was evaluated by estimating the elasticity and the water-holding capacity of the finished products. The elasticity was measured by means of a tester normally used for determining the elasticity of Japanese fish sausage, as illustrated in Figure 1. A strip of sausage (9x9x45 mm^3.) was fixed between (c) and (c') and

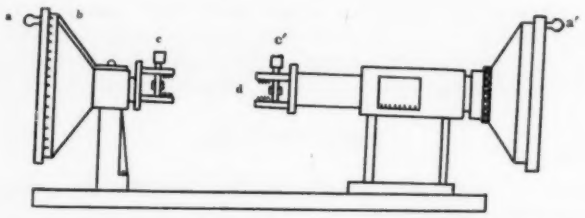

Figure 1. Elasticity tester for sausage. a — Handle for stretching the sample; a' — Handle for twisting the sample; c and c' — Screw for fixing the sample; d — Constant pole.

stretched by turning the handle (a') by 7mm.; then the apparatus was set to work by slowly turning the wheel (a) which can be slid along a scale indicating the twist sustained at any moment by the strip of sausage. When breakage occurred, the wheel was stopped and the degree of twist read at that point. The reading was made on 10 samples of each sausage; the average value was used to indicate the elasticity of the sausage in Figure 6. In addition, a slice (4 mm. in thickness) from the product was set in a frame of 20 mm. width and photographs were taken to record the strain as shown by the stressed surface of the slice. The elasticity of the product was judged in accordance with the photographic evidence.

Water-holding capacity in this report was defined as follows:

$$\text{Water-holding capacity} = \frac{\%\text{ moisture content of meat after cooking at 70° C.}}{\%\text{ moisture content of meat before cooking at 70° C.}} \times 100$$

and it was also judged from the photographic evidence, namely, the appearance of the plane surface of the slice described above.

RESULTS

1. Change of nitrogen extractability

As shown in Figure 2., the amount of the Weber-Edsall-solution-extractable nitrogen from incubated muscle at 20° C. decreased quickly after death of the animals. However, it increased again after this period of decrease and began to show a larger amount of extractable nitrogen at the early stage of putrefaction than it did just following death. After this stage, no further changes in nitrogen extractability could be observed. But when the meat, chilled at 4° C. for a week, was stored at 20° C., the amount of the Weber-Edsall-solution-extractable nitrogen showed a higher value than that from the meat immediately after death. Smaller changes in extractable nitrogen were observed during storage.

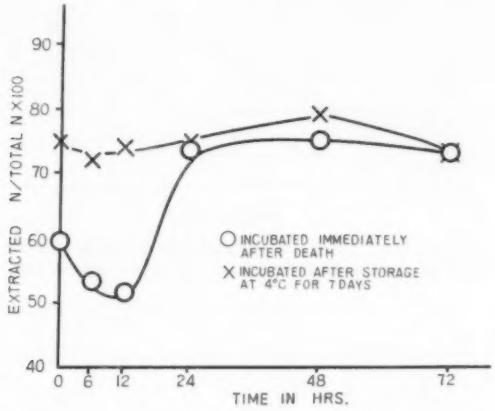

Figure 2. Change of the Weber-Edsall-solution-extractable nitrogen in rabbit skeletal muscle stored at 20° C.

2. Change of pH of muscle

The time-pattern of changes of pH in meat is illustrated in Figure 3. It agrees well with the results published by Bate-Smith and Bendall. From Figure 3 one may note that by prolonged starvation of the animal or by injection of insulin it is possible to maintain a higher pH in the meat during rigor mortis than in the meat under normal conditions. After resolution of rigor, however, all the pH values of the different meat samples increase gradually and can be plotted in a curve that simulates the progress of autolysis and putrefaction.

3. Change of enzymic activity

Since the ATPase activity in muscle homogenate is attributed to the myosin-ATPase *(15)* in the structural proteins of muscle and to the mitochondria-ATPase *(3, 11, 17)*, both are estimated together in the presence of calcium as a modifier (Figure 4). In the case of stored meat, the latent mitochondria-ATPase came to be especially apparent *(12, 14)*; it

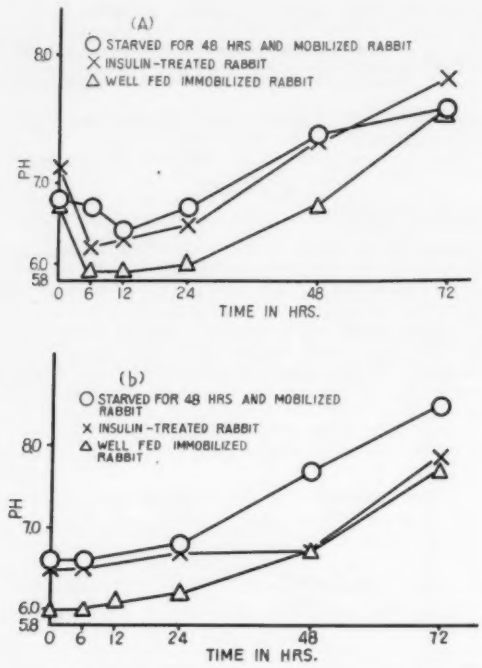

Figure 3. Change of pH in rabbit skeletal muscle stored at 20° C. (A): Incubated immediately after death; (B): Incubated after storage at 4° C for 7 days.

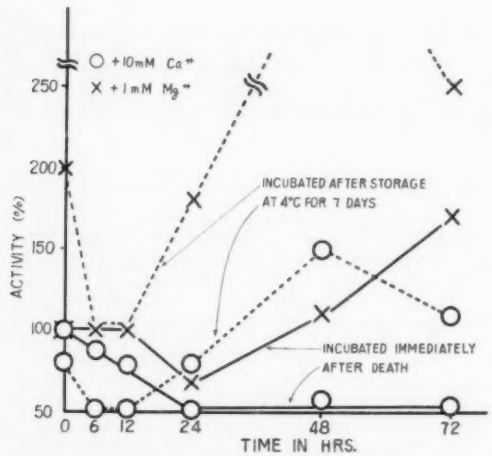

Figure 4. Change of ATPase activity in rabbit skeletal muscle stored at 20° C.

was therefore impossible to detect the inactivation of myosin-ATPase in meat during storage. Hence, EDTA, known either as an extreme inhibitor for mitochondria-ATPase *(18)* or as a strong activator for myosin- or myosin B-ATPase *(13, 23)*, was added to muscle homogenates as a modifier in order to enable observation of the denaturation of the myosin B component in meat. The results obtained are shown in Figure 5. It is clear from the results that the course of inactivation of myosin B-ATPase in meat coincided with that of isolated myosin B-ATPase previously reported by the authors *(26)*; the time-pattern of the inactivation rate of isolated myosin B-ATPase at 20° C. was almost the same whether EDTA or calcium was used as a modifier.

4. Change in the quality of sausage

As indicated in Figure 6, the elasticity of sausage prepared from meat stored at 20° C. decreased considerably as a result of storage for 12 hours after death. A transient rise of

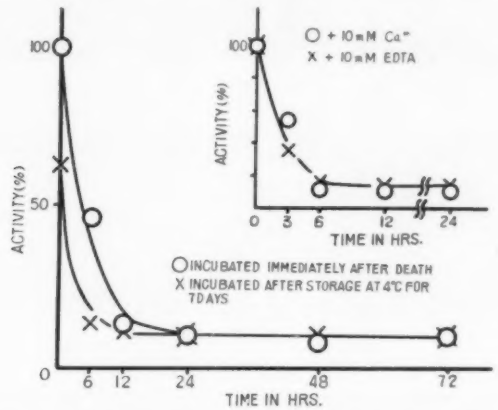

Figure 5. Change of ATPase activity in rabbit skeletal muscle stored at 20° C, in the presence of 10 mM EDTA. The upper figure is inserted for comparison to show the inactivation of isolated myosin B-ATPase during storage at 20° C.

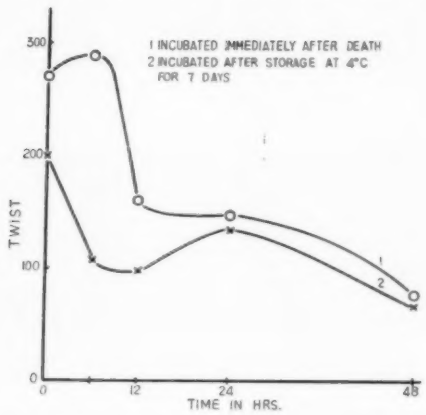

Figure 6. Change of elasticity of sausage from meat material stored at 20° C.

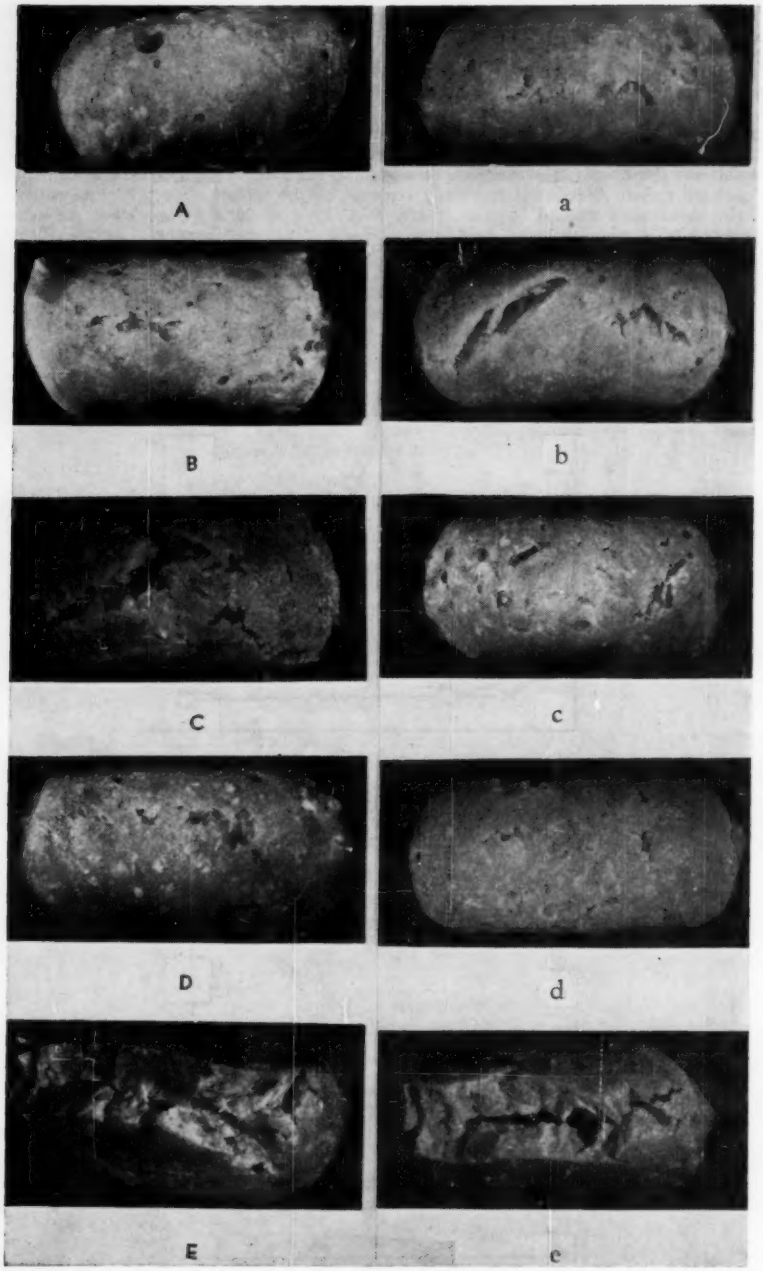

Figure 7. Change of elasticity of sausage from meat material stored at 20° C. Capital letters: incubated immediately after death; small letters: incubated after storage at 4° C for 7 days. A,a: 0 hr., B,b: 6 hrs., C,c: 12 hrs., D,d: 24 hrs., and E,e: 51 hrs. after incubation, respectively.

elasticity in the early stage of storage was observed. This might be due to the formation of actomyosin of high viscosity from myosin and actin resulting from the decomposition of ATP in muscle during storage *(2)*. The recovery of elasticity, however, was observed in the early stage of putrefaction (Figure 7). This phenomenon can also be seen in the data of the experiment graphed in Figure 6 (curve 2). Complete putrefaction caused the complete loss of elasticity (Figure 7). These changes in the elasticity of sausage were observed equally in the meat from normal, starved, or insulin-treated rabbits. Recovery of the elasticity seems to coincide with the increase of nitrogen extractability, as shown in Figure 2. On the other hand, in the case of chilled meat, decrease in elasticity was observed after 6 hours' storage and the recovery of elasticity occurred after 24 hours' storage. Since the extractability of nitrogen showed little change in this case, it is conceivable that the amount of soluble nitrogen had little effect on the elasticity of the sausage. However, when the results shown in Figure 5 were compared with those of Figures 6 and 7, a considerable agreement can be seen between the loss of elasticity of sausage from the meat before the occurrence of putrefaction and the inactivation of myosin B-ATPase in muscle. They are, in short, closely related to each other.

Note, however, in Figure 8 that water liberation occurs in the products prepared from meat of low pH (Figure 8, curve 2); such a phenomenon was not observed in the products illustrated in Figure 8 (curve 1), probably because the pH values of the meat were maintained nearly within the neutral region by use of material obtained by slaughtering insulin-treated animals. Moreover, as shown in Figure 9, typical water liberation was observed in the products prepared from beef *M. semitendineus* in which the pH decreased remarkably after death (Table 1). As well known, physico-chemical properties of myosin B show extreme similarity among mammals. Therefore, from the results mentioned above, support is given to the idea that the water-holding capacity of meat depends considerably upon the pH and that the capacity decreases when the pH approaches the isoelectric point of myosin B.

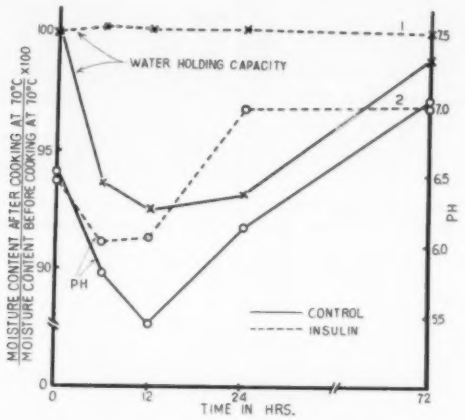

Figure 8. Change of water-holding capacity of rabbit meat stored at 20° C (incubated immediately after death).

5. Microscopic observation

The recovery of elasticity before the onset of putrefaction appears to be due to the structural decomposition of muscle tissue. Suspensions of muscle homogenate from the meat stored at 20° C. were therefore examined microscopically at regular intervals of time, using Olympus Model U.C.E. microscope (X1000). The results obtained are shown in Figure 10.

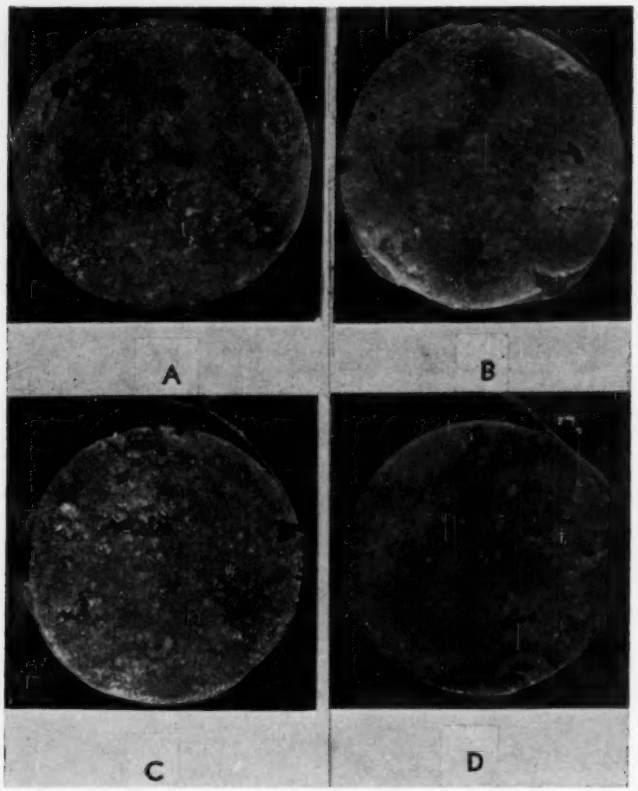

Figure 9. Change of water-holding capacity of beef stored at 20° C (incubated immediately after death). A: 0 hr., B: 6 hrs., C: 24 hrs., and D: 72 hrs. after incubation, respectively.

TABLE 1

Chemical changes of beef M. semitendineus incubated at 20° C. immediately after death

Storage time in hrs.	pH	Soluble nitrogen [1] (%)	ATPase activity [2] mg. P/min./g. protein
0	6.5	56.88	6.79
6	6.1	55.44	——
12	5.5	54.24	2.74
25	5.6	41.19	2.40
48	5.7	50.66	2.41
72	5.8	59.15	2.40
120	7.0	65.25	2.78

[1] Weber-Edsalls' solution extractable N/Total N × 100.
[2] ATPase activity of extracted myosin B. 0.6 M. KCl, 10 mM. EDTA.

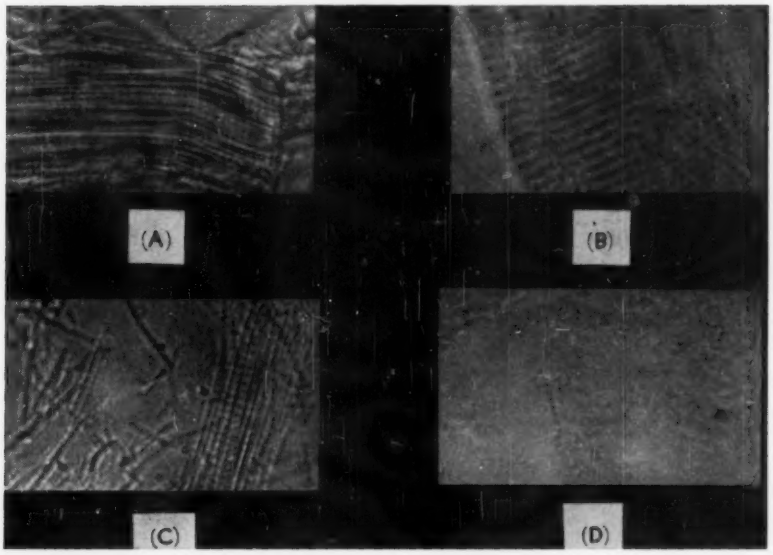

Figure 10. Photomicrographs of muscle homogenates from rabbit meat stored at 20° C. All samples were suspended in 0.1 M KCl solution. A,B: 0 hr., C: 24 hrs., and D: 70 hrs. after death, respectively.

Muscle fibers in fresh meat were in the form of masses of bundles which were maintained intact even through the homogenizing process; the fibers after 24 hours' incubation began to loosen and to break easily. The protein in solution now acted as a sort of cementing substance for these loosened fibers and in turn caused the apparent recovery of elasticity. In the putrefaction stage, however, muscle fibers were dissolved, resulting in loss of their original structure.

6. Experiments on myosin B

The results with respect to the inactivation of ATPase activity of muscle homogenate suggested that the elasticity of sausage depended principally upon the denaturation of myosin B in muscle. Experiments were carried out to clarify this problem further and an examination of the properties of myosin B from beef *M. semitendineus* during storage at 20° C. was undertaken. As shown in Table 1 and Figure 11, the rates of inactivation of myosin B-ATPase and the salting-out curves of this protein extracted from stored beef coincided almost exactly with those of isolated myosin B stored at 20° C, independently of the remarkable decrease of the pH in the meat and of the changes in nitrogen extractability. It was evident from these facts that denaturation of myosin B occurred in the muscle during storage.

DISCUSSION

Many studies on meat quality have been based upon the water-holding capacity of meat. Some of these investigations *(6, 21)* suggest that the most essential substance in the curing agents which affect this property is sodium chloride and that the optimum concentration of sodium chloride in meat is 2.5-3.0% (W/W). In other studies *(1, 4, 7, 8, 22, 24, 25)* on the effects of various cations on the water-holding capacity of meat it is reported that

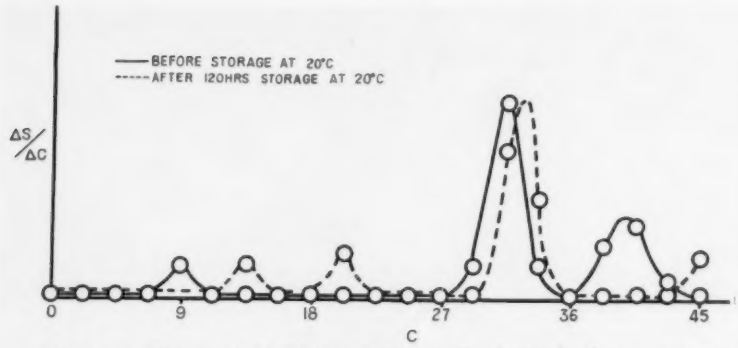

Figure 11. The derived salting-out curves. S: the extinction at 750 mμ; C: %(V/V) of saturated ammonium sulfate solution. The solid line and the dotted one represent, respectively, the curves of extracted myosin B from beef M. semitendineus before and after storage at 20° C. for 120 hrs.

actomyosin in muscle proteins may play an important role in the phenomenon. It is considered that the most substantial factor effecting this phenomenon may not be the *amount* of extractable nitrogen but the *electric charge* in the protein molecule.

It is suggested by these reports that the physico-chemical changes of myosin B molecule originates at least partially in the change in the quality of meat during storage.

W. Partmann *(19)* investigated the effects of practical factors in the sausage manufacturing process on the apyrase system of the muscular tissue and obtained some important and interesting results. However, he did not recognize clearly the denaturing process of myosin B in meat during storage; his work was carried out, it would appear, without due regard for the mitochondria-ATPase system. The inactivation of myosin B-ATPase activity in muscle homogenate was investigated in the presence of EDTA in the same manner as in the detailed study on the denaturation of isolated myosin B during storage *(26)*. It seems that a first-order course of ATPase inactivation in the denaturation process of the protein coincided in considerable degree with the decrease in the elasticity of sausage during storage before the putrefaction stage and that myosin B plays an important role in elasticity. Furthermore, the fact that the changes in the salting-out behavior of myosin B extracted from beef *M. semitendineus* during storage at 20° C. coincided perfectly with those of isolated myosin B stored at 20° C. *(26)*, supports the conclusion stated above.

However, as discussed previously, the water-holding capacity of meat depends considerably upon the pH, especially in the region of pH values below 6. It is supposed that the change in this property is caused by the approach of the pH of the meat to the isoelectric point of myosin B, as usually observed in the proteins.

Although, during progress to the putrefaction stage, some apparent recovery of elasticity was observed, the results of microscopic observation suggest that this phenomenon is caused mainly by the structural decomposition of muscle through the dissolving process.

In this paper, the authors have discussed the biochemical and physical properties of myosin B, since it was supposed that the salt-soluble proteins in meat play a decisive role under the condition of high salt concentration as in the meat products. In the case of rabbit skeletal muscle, however, actomyosin constitutes 52–54% of the total muscle proteins *(9)*. The remaining portion represents the protein fractions soluble in meat juice, with the exception of a small quantity (0.2–0.4%) of connective tissue. Attention must therefore be paid to the fact that skeletal muscle contains 30–45% of proteins other than actomyosin, and that these proteins may also contribute to the formation of the physical properties of the experimental sausages, especially, in the recovery stage of the elasticity of sausages as indicated by the authors. Further research will have to be carried out to define the role of these proteins.

SUMMARY

Investigations were conducted on the effect of storage of meat materials at 20° C. on the nitrogen extractability, the pH value, and ATPase system of muscular tissue and on the quality of sausage.

During the post mortem storage of the meat at 20° C., the Weber-Edsall-solution-extractable nitrogen from the muscle decreased transiently and then increased gradually through the process to the putrefaction stage. When the meat chilled for 7 days was incubated under the same condition, less change of nitrogen extractability was observed than before.

The pH of muscle was greatly influenced by the condition of the animals before slaughter. Prolonged starvation of animals or injections before slaughter resulted in the maintenance of the pH near the neutral region.

The course of inactivation of myosin B-ATPase in the presence of EDTA coincided perfectly with that of myosin B-ATPase in the presence of calcium. Only in the presence of EDTA as a modifier was the course of inactivation of myosin B-ATPase in muscle homogenate during long storage clearly observed, though the mitochondria-ATPase in muscular tissue, when calcium was used as a modifier, appeared at the same time. As a result of the inactivation of myosin B-ATPase in muscle, the enzymic activity of myosin B in muscle was found to be inactivated quickly during storage at 20° C.

The quality of sausage prepared from stored meat samples was examined by testing the elasticity and the water-holding capacity of the products. It seemed that the elasticity of the products decreased mainly in correspondence with the denaturation of myosin B in muscle, but irrespective of the nitrogen extractability.

On the other hand, water-holding capacity was affected considerably by the pH of the meat, especially in the case of pH values below 6.0.

Structural changes of muscle homogenate were observed microscopically. It was noted that the mass of bundles in the early stage of storage disintegrated and finally were dissolved.

The change of the enzymic activity and the salting-out behavior of myosin B extracted from stored meat were investigated at regular intervals of time during storage at 20° C. The results obtained coincided completely with those observed in isolated myosin B.

Acknowledgment

The authors cordially thank Dr. Y. Tonomura of the Institute for Catalysis, this University, for his warm support and valuable advice both during the course of our work and the preparation of the manuscript. Our thanks are also tendered to Mr. C. Mochizuki and Mr. T. Nagahashi, this laboratory, for their help in the experiments. This study has been supported in part by a grant-in-aid from the Scientific Fund of the Ministry of Education to the Research Group at work on the "Protein Chemistry of Meat Products."

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THE EFFECT OF HOUSEHOLD TABLE SALT ON THE WHIPPING QUALITY OF EGG WHITE SOLIDS*

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(Manuscript received June 9, 1958)

In the past few years the production of egg white solids has increased from an estimated four million pounds in 1951 *(9)* to nine and one half million pounds in 1957 *(11)*. Many new uses have been developed for the product. Egg white solids are extensively and very satisfactorily used in cake mixes and other food mixes. When reconstituted directly with water the egg albumen lumps and will dissolve only after extensive stirring. It was thought that some additive, perhaps an ingredient commonly used in products made with egg whites, might influence the ease of reconstitution. Any additive used to improve the reconstitution of egg white solids must not impair the foaming ability and should improve it. Since salt is an ingredient commonly used in most food products it seemed an obvious substance to test. In this study the influence of salt on the ease of reconstitution of egg white solids and on the whipping qualities and stability of these reconstituted whites was tested.

Considerable research has been reported on the foaming properties of liquid egg whites. It is logical to assume that the same factors which influence the whipping quality and stability of the liquid whites would also affect reconstituted egg white solids. Barmore *(1)* stated that acid and acid salts increased the foam stability of liquid egg. Slosberg, Hanson, Stewart and Lowe *(8)* indicated that the rate of beating had some effect on foaming action. Henry and Barbour *(4)* mentioned that time of beating and temperature of egg albumen were important to foam formation and stability. McKellar and Stadelman *(7)* found that larger volumes were matched in many cases by increased drainage, substantiating the theory that maximum volume does not result in maximum stability. Jordon and Whitlock *(5)* reported that treatment of fresh egg albumen with salt resulted in a small, but significant lowering of the apparent viscosity.

A few studies on the characteristics of reconstituted dried egg whites have been reported. Joslin and Proctor *(6)* stated that dried egg white had essentially the same whipping characteristics as fresh egg white. Carlin and Ayres *(3)* used an enzyme preparation in the production of a glucose-free dried albumen, and tested the whipping properties of those rehydrated egg whites in angel cakes. They found that pan dried albumen rehydrated readily and was free of any objectionable odor or flavor. Barnwort and Ayres *(2)* reported that in egg solids containing moisture of from 1.5% to 6% there was an improvement in specific gravity of the meringue during storage at either 50 or 60° C.

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* Journal Series Paper No. 1877, approved by the Director of the Missouri Agricultural Experiment Station.

Experimental Procedure — Equipment and Materials

Commercial egg white solids, both pan dried and spray dried, were reconstituted using distilled water containing varying amounts of household table salt (iodine free). Both types of egg albumen were obtained from a commercial drying plant[b] immediately after drying. The albumen was kept tightly sealed throughout the tests to avoid possible moisture loss or gain. Table salt was added to 11 series of samples; one series was tested without salt. Each series consisted of 10 replicates. Although salt levels ranged from 0-9 g., the flavor acceptance level was not above 1.5 g. per 2 egg whites as was established by a panel of judges sampling the various salt levels in soft meringue.

A torsion balance was used for weighing samples of egg solids and salt, and for weighing and determining specific gravity. A 1956 Kitchen-aid food mixer, model 3-C, (with reproducible settings to 10 speeds) with glass bowl was used in whipping all samples. A 100 ml. graduate cylinder was used for reconstitution which was accomplished in pyrex custard cups. The index of volume was determined by use of a millimeter measuring stick. Metal containers measuring 6.5 mm. in diameter and 7.3 mm. inside depth (about 1 cup capacity) with a centered bottom opening measuring 0.4 mm. in diameter and sealed with adhesive tape were used to measure all egg foam. Ten milliliter graduate cylinders were used in leakage determinations.

Physical Tests

Both pH and moisture determinations were made on the egg albumen prior to tests on whipping and stability. The pH of the pan dried samples was found to average 5.83 with a range of 5.80-5.88; that of the spray dried average was somewhat higher, 7.31 with a range of 7.28-7.35. Moisture tests showed the average pan dried albumen sample to contain 15.0% moisture with a range of 15.0-15.1, whereas the average spray dried sample contained 8.65% moisture with a range of 8.60-8.70%.

Samples of the egg white solids were weighed and reconstitution ratios as given by U. S. Department of Agriculture, Home and Garden Bulletin No. 50 *(10)* were modified on the basis of moisture determinations made in our laboratory. The distilled water, at room temperature, was poured over a specific quantity of salt in a pyrex custard cup and the salt was stirred briefly into solution. The egg white solids were then sprinkled onto the surface of the salt water solution and stirred at frequent intervals until dissolved. The temperature was recorded and the reconstituted egg whites were transferred to the mixer bowl.

Reconstitution ratios used (2 egg white equivalent)

	Egg White Solids	Distilled Water
Pan Dried Albumen	8.25 g.	49 ml.
Spray Dried Albumen	7.30 g.	47 ml.

All egg whites were whipped at speed 8 (beating-whipping) until the whites were stiff enough to form soft, moist peaks (from 1 minute, 30 seconds to 2 minutes, 30 seconds depending on salt level). The foam was then quickly, yet gently, smoothed to form a level surface in the mixer bowl. The mm. measure was then inserted at 3 separate points in the foam near the center of the bowl and the average reading recorded. A rubber spatula was used to fill the metal container with foam and a small metal spatula to ease out air pockets and level off the foam. This container of foam was quickly weighed to determine specific gravity, the adhesive cover was removed from the opening and the container placed on the ringstand to measure leakage. Each sample was allowed to stand for 30 minutes and a record was kept of the time when breakdown began and of the total amount of liquid collected. A stop watch was used to time all procedures.

RESULTS AND DISCUSSION

It was noted during this experiment that the pan dried albumen was more quickly and easily reconstituted than the spray dried. It was not known

[b] Henningsen, Inc. — Springfield, Mo.

whether the slight granular consistency of the pan dried or its higher moisture content caused this desirable action. It is quite possible that it was a combination of the two. The presence of salt in varying concentrations seemed to have little or no effect upon ease of reconstitution.

Table 1 compares results of the various tests obtained with the pan dried and spray dried egg albumen. This table is a condensed version of the record data chart and shows the averages of 10 samples at each salt level.

Volume. The index of volume (Table 1 and Figure 1) of egg foam

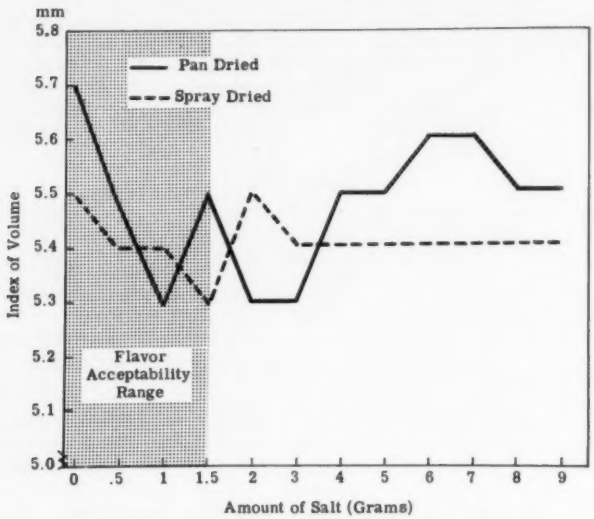

Figure 1. The effect of table salt upon the index of volume of both pan dried and spray dried egg albumen.

from the pan dried albumen was generally greater than that from the spray dried, and showed more variability with the salt level. Within the flavor acceptability range, the presence of salt decreased the volume of the egg foam in both kinds of egg albumen. Although above the acceptable flavor range there was generally an upward trend, the volume never equaled that of the no-salt samples. From results obtained in the study it must be concluded that the salt does not increase the volume of foam from dried egg albumen.

Specific gravity. Specific gravity, as measured, appeared to increase steadily as salt was added (Table 1 and Figure 2). However, when corrections were made for the weight of salt in the foam, the increase in specific gravity was marked only with the addition of 0.5 grams of salt. The corrected specific gravity was fairly constant after the first addition of salt. The difference in specific gravity between the two types of dried egg albumen was small at all salt levels.

Leakage. Results of the study of table salt on the stability of dried egg albumen foam (Table 1 and Figure 3) showed that within the flavor ac-

TABLE 1

Effect of addition of salt on the whipping qualities of pan and spray dried albumen *

Salt (Grams)	Pan dried egg albumen								Spray dried egg albumen							
	Whipping time		Volume (mm)		Specific gravity		Leakage (ml)		Whipping time		Volume (mm)		Specific gravity		Leakage (ml)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
0.0	1'30"	1'30"-1'30"	5.7	5.6-5.8	.082	.078-.088	2.2	1.2-4.0	1'29"	1'20"-2'00"	5.5	5.4-5.5	.087	.085-.090	3.3	2.0-4.5
0.5	1'59"	1'45"-2'00"	5.5	5.4-5.6	.093	.090-.097	3.0	2.1-4.1	2'00"	2'00"-2'00"	5.4	5.4-5.5	.093	.091-.096	4.6	2.0-6.0
1.0	1'54"	1'30"-3'00"	5.3	5.2-5.5	.094	.086-.104	5.3	4.7-6.5	2'30"	2'00"-2'15"	5.4	5.3-5.5	.093	.088-.098	4.4	3.0-5.1
1.5	2'15"	2'15"-2'15"	5.5	5.5-5.5	.095	.093-.098	3.1	2.2-4.1	2'50"	2'00"-2'30"	5.3	5.1-5.4	.097	.090-.108	5.3	1.8-7.8
2.0	2'16"	2'15"-2'30"	5.3	5.2-5.5	.098	.092-.102	3.7	2.5-5.2	2'15"	2'15"-2'15"	5.5	5.4-5.5	.094	.089-.098	4.1	3.0-5.0
3.0	2'16"	2'15"-2'30"	5.3	5.2-5.4	.099	.093-.105	3.6	2.5-5.6	2'16"	2'15"-2'30"	5.4	5.3-5.5	.098	.094-.101	3.9	2.8-4.9
4.0	2'15"	2'15"-2'15"	5.5	5.4-5.5	.100	.096-.106	2.5	1.3-4.8	2'15"	2'15"-2'15"	5.4	5.3-5.5	.097	.093-.101	3.1	1.7-4.0
5.0	2'15"	2'15"-2'15"	5.5	5.5-5.6	.096	.095-.100	1.5	0.8-2.7	2'18"	2'15"-2'30"	5.4	5.3-5.4	.101	.100-.105	3.2	2.2-4.3
6.0	2'15"	2'15"-2'15"	5.6	5.5-5.7	.101	.098-.106	1.7	1.1-2.2	2'30"	2'30"-2'30"	5.4	5.3-5.5	.103	.099-.107	1.9	0.7-2.8
7.0	2'15"	2'15"-2'15"	5.6	5.5-5.7	.102	.098-.106	1.2	0.7-1.8	2'30"	2'30"-2'30"	5.4	5.3-5.4	.104	.101-.108	1.3	0.5-2.8
8.0	2'15"	2'15"-2'15"	5.5	5.5-5.6	.105	.100-.111	1.0	0.5-1.8	2'30"	2'30"-2'30"	5.4	5.3-5.4	.105	.101-.111	0.4	0.1-0.8
9.0	2'15"	2'15"-2'15"	5.5	5.4-5.6	.106	.102-.111	0.6	0.2-1.2	2'30"	2'30"-2'30"	5.4	5.3-5.5	.107	.103-.110	0.0	0.0-0.1

* 10 samples at each salt level.

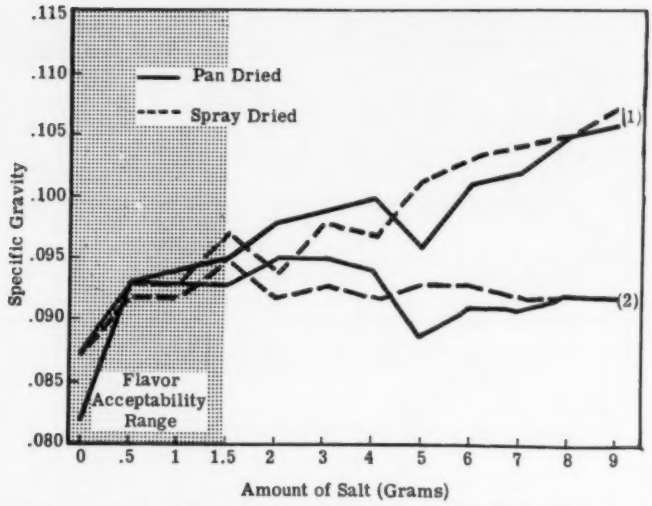

Figure 2. The effect of added salt on the specific gravity of foam from pan dried and spray dried albumen (1) as observed, and (2) as corrected for weight of salt.

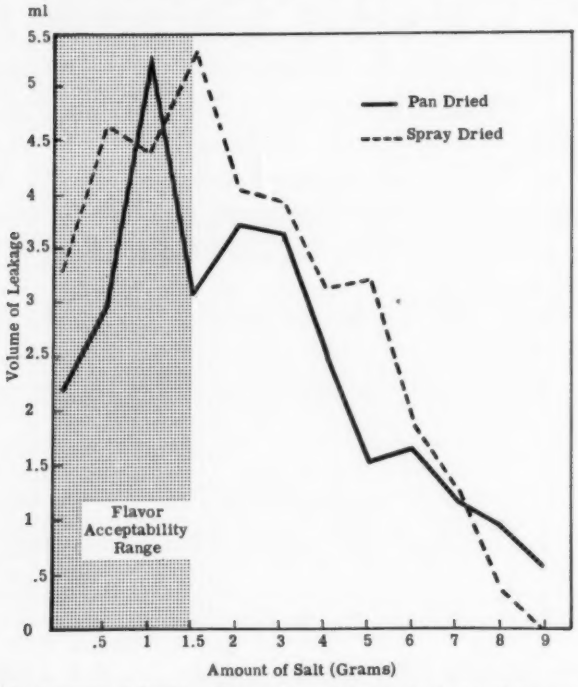

Figure 3. Amount of liquid collected from the 30 minute breakdown of both pan dried and spray dried egg foam with various levels of salt.

ceptability range, as the salt level was increased the leakage tended to increase. There was a sharp rise in the amount of leakage with the first two or three salt additions, then a steady trend toward decreased leakage throughout the higher salt levels. In the spray dried foam the 9 g. salt level appeared to completely stabilize the foam over the 30 minute test period, but this salt concentration is too high to be of practical importance. The pan dried albumen pattern of leakage was very similar to the spray dried albumen.

Whipping time. The most marked increase in whipping time (Table 1 and Figure 4) was about 30 seconds or 25% with the first 0.5 g. added

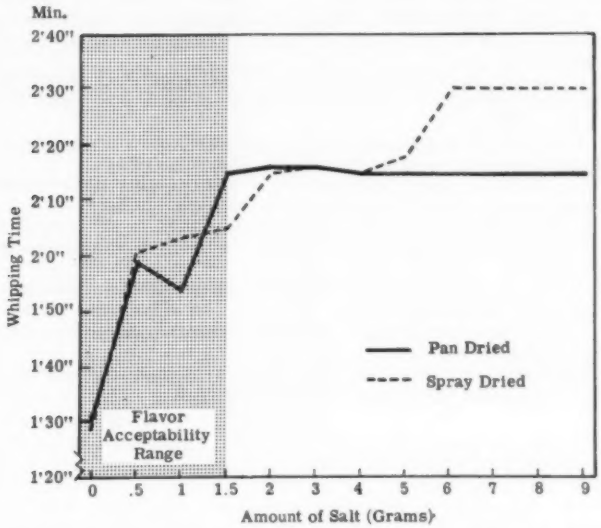

Figure 4. The effect of salt on the whipping time required to form soft, moist peaks in the foam of reconstituted pan dried and spray dried samples.

salt in both types of dried albumen. At the 1.5 g. salt level the pan dried reached its high and held this plateau throughout. The spray dried albumen followed a similar pattern, but reached an even higher plateau at the 6-9 g. salt level.

SUMMARY

Selected levels of household table salt were used to test their effect on the whipping quality and stability of foam from pan dried and spray dried egg albumen. These concentrations of salt seemed to have little effect upon ease of reconstituting the dried egg albumen. There appears to be little beneficial effect of salt upon the foam of dried egg albumen within the flavor acceptability range. Although a slight increase in specific gravity was noted the salt apparently decreased both foam volume and foam stability within this range. Salt concentrations, above the acceptable flavor range, had little effect on corrected specific gravity but did tend to stabilize the foam until virtually no breakdown occurred. Although a considerable variation in vol-

ume was recorded throughout, there was a decrease in volume as compared to the 'no-salt' sample. In general, it was necessary to lengthen whipping time as the salt concentration in the foam was increased.

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VARIABILITY IN TENDERNESS OF CHICKEN[a]

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(Manuscript received May 31, 1958)

One of the difficult problems in food research is the variability of the food material itself. This is especially true with any food which cannot be ground and mixed without losing its identity. It has been demonstrated *(2)* that chickens hatched from eggs laid by the same hen will vary less in body weight than chickens from eggs from different hens within the same flock. This study was designed to test whether this decrease in variability would carry over to losses during cooking and to tenderness of the cooked chicken.

PLAN AND PROCEDURE

The chickens were hatched from eggs collected from a mass mated flock of New Hampshires. Previous studies on heritability of body weight in this population indicated that about 10 to 25% of variability might be eliminated by restricting experimental comparisons to offspring of the same hen. All the chickens were fed and handled alike. Testing was started when the chickens were 8 weeks old, and continued for 10 days, giving an age range from 8 weeks to 9 weeks 4 days within each hatch. Data were collected from two replicated hatches.

Within a hatch, material tested each day consisted of 6 birds, 3 of each sex. Within each group of three, 2 chickens were from eggs laid by the same hen, making these 2 chickens half-sibs. The third chicken was from an egg laid by a different hen. The experiment was designed to permit comparison of the variation between birds from different hens with that among birds from the same hen.

Preparation and sampling. The chickens were slaughtered and held under refrigeration for 24 hours before cooking. They were roasted, breast side down, in a rotary hearth oven, at 150° C. Thermometers[b] were inserted in the breast area so that the bulb of the thermometer was between the *pectoralis major* and *pectoralis minor* muscles. The birds were roasted to a breast temperature of 80° C. Appropriate weights were recorded for determination of cooking losses. The liquid which accumulated in the body cavity during cooking was drained into the pan and included in the dripping losses.

The cooked birds were cooled to 40° C. before sampling. Three slices one-fourth inch thick were cut from the anterior portion of the *pectoralis major,* starting one inch from the anterior end and cutting toward the posterior. A one-inch piece was cut from the side of each slice nearest to the keel bone. These standard-size pieces were served to the scoring panel for estimation of tenderness by recording the number of chews necessary to masticate the sample. The rest of the slice was scored for tenderness on a scale ranging from 10 *(extremely tender)* to 1 *(extremely tough).* The remainder of the *pectoralis major* was used for mechanical estimation of tenderness by shearing on the Warner-Bratzler shear machine.

Chemical analyses. It seemed of interest to check whether or not the amount of nitrogen extractable by buffer solution would show any relation to tenderness, as had been reported for beef by Wierbicki, Kunkle, Cahill and Deatherage *(3).* Accordingly, the pieces of the *pectoralis major* remaining after shearing were ground, aliquots utilized for buffer extraction, and the remainder packaged and frozen for later analysis for moisture, fat, and total nitrogen.

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[a] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, May 28, 1958.

[b] With this kind of material thermometers are less likely to be displaced than thermocouples and have, moreover, a larger temperature sensing area.

The method used for buffer extraction of nitrogen was essentially that outlined by Wierbicki, *et al. (3)*. The buffer solution consisted of potassium chloride, potassium hydroxide and citric acid, to give a solution of pH 5.60 ± 0.05, ionic strength 0.48, containing 0.22 M KCl. The extracts were prepared on the day the chickens were cooked, and were frozen for later analysis. The nitrogen in the buffer extract was determined by micro-Kjeldahl analysis. Moisture was determined on the ground meat by drying in a vacuum oven at 70° C. and 25-28 mm. vacuum, for 16 hours. Fat was determined by extracting the ground, dried meat with petroleum ether, using the Soxhlet apparatus. Total nitrogen in the meat was measured by Kjeldahl analysis.

RESULTS

Averages of the data are listed in Table 1 by hatch and by sex. These figures illustrate the size of the values obtained. The F-values from analysis of variance are given in Table 2. These show the following significant differences: (1) sex difference in carcass weight — males heavier than females; (2) difference among daily groups of three in weight of *pectoralis major,* percent total cooking loss, and percent evaporation loss — variations were irregular and could not be attributed to differences in age of chickens within a hatch; (3) greater uniformity among pairs than among unrelated birds in carcass weight and in percent drip losses; and (4) marked differences among individuals within pairs. The last comparison (4) could be made only where measurements on the right and left sides of the same bird were available.

Estimates of variance components for half-sibs, individuals, and within individuals (right versus left sides of same carcass, where available) are shown in Table 3, as mean squares and as percent. On the percentage basis, the data indicate that the use of maternal half-sibs may reduce experimental errors in measurements of carcass weight by about 35%, and of dripping losses by about 30%. For the various measures of tenderness, reduction of experimental error was effected by making comparisons between the right and left sides of the same individual, rather than by use of half-sibs.

The basis for the successful reduction of error by the use of maternal half-sibs for some measures, but failure with others is illustrated in Table 4. This gives the carcass weights and average tenderness scores for the female chickens. In 65% of the cases, the carcass weights for the paired (half-sib) chickens were closer to each other in value than were either one of them to the weight of the chicken from a different mother. However, the opposite picture was found in the tenderness scores — in 80% of the cases there was less difference between the score for the "different mother" chicken and the score for one of the two half-sibs than between the scores for the two half-sibs.

Pertinent correlation coefficients between various measurements are given in Table 5. The correlation between tenderness score and percent nitrogen extracted by the buffer solution was high enough to be statistically significant, but too low to indicate decided usefulness of this means for measuring tenderness of chicken. The correlations among the

TABLE 1

Average data for different hatches and different sexes

Factor	Hatch		Sex	
	I	II	Male	Female
Carcass weight, grams	717.89	743.34	822.90	638.33
Pectoralis major weight, grams	36.55	39.34	41.74	34.15
Total cooking loss, percent	22.00	21.55	21.89	21.65
Drippings loss, percent	3.41	3.32	3.33	3.40
Evaporation loss, percent	18.59	18.24	18.57	18.25
Tenderness score, pectoralis major (max. = 10)	6.7	7.0	7.0	6.6
Number of chews, pectoralis major	38.1	38.6	36.1	40.5
Shear, pounds (1st 2 cuts, pectoralis major)	11.15	9.73	10.05	10.83
Percent nitrogen extractable by buffer	—	—	9.70	9.59

other measures of tenderness were quite high, especially between tenderness scored on a 10-point scale and tenderness evaluated by counting the number of chews required to masticate a standard-sized sample. Shear values based on the two cuts most nearly adjacent to the samples for scoring correlated better with the tenderness scores than did the shear values based on all the readings from the pectoralis major. Conversion of the shear data by dividing by the weight of the muscle increased somewhat the correlation coefficient between shear and tenderness score, but not between shear and number of chews. This conversion was made in an attempt to remove the influence of muscle size,

TABLE 2
F-values, analysis of variance

Source of variance	Degrees of freedom	Carcass weight	Weight of pectoralis major	Cooking Losses			Tenderness measures, pectoralis major		
				Total	Drip	Evaporation	Score	No. of chews	Shear (1st 2 cuts)
Hatch	1	24.42	8.84	3.29	< 1	< 1	< 1	< 1	7.13
Sex	1	1284.38*	65.65	< 1	< 1	< 1	1.15	2.22	2.14
Hatch-Sex Interaction	1	< 1	< 1	< 1	< 1	< 1	2.66	2.00	< 1
Daily group of three	36	1.48	3.77**	1.71*	1.12	2.03*	1.40	1.25	< 1
Pairs vs. unrelated individuals	40	2.14*	1.23	< 1	1.82*	< 1	< 1	< 1	< 1
Individuals within pairs	40	——	10.08**	——	——	——	7.15**	6.56**	4.90**

* $p < 0.05$
** $p < 0.01$

TABLE 3

Estimates of variance components between half-sibs, between individuals, and within individuals.

Measure	Variance components expressed as					
	Absolute value			Percent		
	Half-sib	Between individuals	Within individuals	Half-sib	Between individuals	Within individuals
Carcass weight	1554.35	2737.91	—	36.2	63.8	—
Pectoralis major weight	1.18	9.36	2.06	9.3	74.3	16.4
Total cooking losses	negative	12.02	—	0.0[1]	100.0	—
Drippings losses	0.29	0.71	—	29.0	71.0	—
Evaporation losses	negative	11.61	—	0.0[1]	100.0	—
Tenderness score	negative	1.80	0.59	0.0[1]	75.3	24.7
Number of chews	negative	147.25	53.01	0.0[1]	73.5	26.5
Shear, 1st 2 cuts	negative	10.02	5.14	0.0[1]	66.0	34.0

[1] For prediction purposes, negative components are assumed to be zero.

TABLE 4

Raw carcass weights and average tenderness scores for female chickens

Hatch	Daily Group	Carcass Weight, g.			Average tenderness score		
		Same Mother		Different Mother	Same Mother		Different Mother
		1	2		1	2	
I	1	644.0	652.5	589.5	8.0	8.2	8.2
	2	603.0	674.0	636.5	6.5	7.8	4.4
	3	646.5	600.0	574.5	7.5	5.5	7.0
	4	620.5	628.0	634.0	6.4	4.8	6.8
	5	739.5	707.0	610.0	7.8	6.5	8.0
	6	621.0	637.0	692.0	3.7	8.3	5.7
	7	601.5	575.0	733.5	5.6	6.5	6.2
	8	517.5	623.0	742.0	7.8	7.4	7.5
	9	516.5	574.0	711.0	6.3	4.2	4.5
	10	533.0	495.5	559.0	6.8	7.5	9.0
II	1	582.0	540.0	553.0	6.0	5.2	8.2
	2	483.0	585.0	595.0	6.0	5.6	6.2
	3	680.0	655.0	606.0	3.8	8.0	7.7
	4	694.0	721.0	577.5	7.3	6.8	6.5
	5	609.0	665.5	636.5	8.5	5.0	7.0
	6	665.0	661.0	779.5	5.8	4.8	7.5
	7	625.0	649.0	700.5	5.0	9.3	7.2
	8	639.5	651.5	695.5	5.0	8.8	7.8
	9	799.0	751.0	557.0	6.0	7.0	5.7
	10	761.0	788.5	703.5	6.0	8.5	5.8

since the shear readings were taken across the entire muscle rather than on a piece with a uniform cross-section. It is possible that conversion of the shear data to a basis of standard cross-section area might be more effective than the weight basis. However, the area figures were not recorded for this experiment.

It has been found in some cases that length of cooking time and fat content of the muscle will affect the tenderness. These were checked, but the correlation coefficients were found to be rather small. The correlation between tenderness score and cooking time was highly significant statistically, and negative rather than positive. The cooking times averaged 12.4 minutes per 100 g. (56.2 minutes per pound), and ranged from 4.3 to 21.3 minutes per 100 g. (19.4 to 96.6 minutes per pound). Rather wide variations in cooking time, especially when products are cooked to the well-done stage, are not uncommon. A convenient recent example is contained in the report of Cover *et al. (1)* wherein the

TABLE 5

Coefficients of correlation between various measures on chickens

Factors being compared	Correlation coefficients
Average tenderness score × % nitrogen extracted by buffer	0.37*
Average tenderness score × number of chews	− 0.94**
Average tenderness score × shear (cuts 1 and 2 on pectoralis major)	− 0.71**
Average tenderness score × shear (cuts 1 and 2 on pectoralis major per 100 g. muscle)	− 0.77**
Average tenderness score × shear (all cuts on pectoralis major per 100 g. muscle)	− 0.64**
Number of chews × shear (cuts 1 and 2 on pectoralis major)	0.75**
Number of chews × shear (cuts 1 and 2 on pectoralis major) per 100 g. muscle)	0.72**
Average tenderness score × cooking time (min/100 g.)	− 0.29**
Average tenderness score × % fat (wet basis)	− 0.39*
Average tenderness score × % fat (dry basis)	− 0.37

* $p \leq 0.05$
** $p \leq 0.01$

broiling time of bottom round steaks cut to standard thickness was noted to vary from 60 to 105 minutes. Apparently, within this time range, there was a tendency for the chickens requiring shorter cooking times to be more tender (higher tenderness score).

SUMMARY

A study was made to see if the decrease in variation in body weight obtained by using chickens hatched from eggs laid by the same hen instead of chickens from eggs laid by different hens within the same flock would also exist in cooking losses and in tenderness of the chicken meat. With the exception of percent dripping losses, use of maternal half-sibs did not decrease the variation in cooking losses and tenderness. The use of duplicate determinations on the right and left sides of the same carcass was a much more effective method of reducing experimental error in the tenderness measurements than was the use of maternal half-sibs.

The correlations between the various tenderness measurements — score, number of chews, shear and percent nitrogen extractable by buffer — were quite high with the exception of the percent extractable nitrogen. This latter attribute was not particularly effective as a means of predicting tenderness in young chickens.

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APPLICATION OF NUCLEAR MAGNETIC RESONANCE IN MEATS RESEARCH[a]

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(Manuscript received May 31, 1958)

The principle of nuclear magnetism of hydrogen has been well established *(1, 5, 7, 13)* and this property of the hydrogen molecule makes possible the quantitative measurement of hydrogen in the liquid *(12, 16, 18)* and solid states *(2, 10, 16)*. In the present case the nuclear magnetism of hydrogen atoms of the water molecule are under consideration.

Briefly, the measurement of water within a sample is dependent upon the ability of the hydrogen nuclei to precess when placed in a magnetic field. Pauli *(7)* first suggested that the nuclei of certain atomic species possess the properties of angular momentum and magnetic moment. Angular momentum is a vector quantity as measured by quantum mechanics employing Planck's constant. The magnetic moment is related to the angular momentum of a species and is measured in units of the nuclear magneton. These units are a measure of the energy level and can be defined as the gyromagnetic ratio. This ratio is unique for any given atomic species. When a sample containing nuclei of a species possessing a gyromagnetic ratio is placed in a magnetic field, the nuclei will align themselves according to the field force. At the proper field force, the nuclei will precess or wobble on their axes. If R-F energy is applied as this phenomena occurs there will be a change in the energy levels within the nuclei. The amount of absorbed R-F energy is dependent on the number of the nuclei that are precessing. This total amount of absorbed R-F energy can be detected, amplified, and measured electronically *(2, 5, 6, 7, 10)*.

Since the water content of meat products is of obvious economic importance to the meat processor and because of the inability of present methods to measure this water content rapidly and continuously, a study of the application of NMR methods on meat products was begun in May of 1956. This new approach to the determination of water in meat may provide a rapid, non-destructive method, as opposed to the conventional, time-consuming, heat-drying methods, during which the physical structure of the sample is altered.

In reviewing the information concerning NMR spectroscopy, it appeared necessary to correlate the signal obtained from a reference meat sample with its known moisture content as determined by conventional heat-drying methods *(11)*. It was the purpose of our work to determine whether moisture measurement of meat could be accomplished by this method. The results of preliminary tests indicated that a signal could be obtained and was reproducible. After this fact was established, it became desirable to investigate

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the inherent components in meat which give rise to an NMR signal. The following considerations were established as test objectives:

1. What basic components of the meat sample being measured contribute to the signal obtained from the instrument?
   a. Is the signal representative of total water in the sample?
   b. Does the signal portray free water only?
   c. Does fat in the sample contribute to the signal?
2. What other factors influence the signal?

EXPERIMENTAL METHODS

The instrument employed for these tests was one which was developed at the Southwest Research Institute under the sponsorship of the Corn Industries Research Foundation and Clinton Foods, Inc. *(11)*. It consists of a 1250 Gauss magnet, a signal detector, an amplifier phase detector, a reference modulation sweep generator, power supply and automatic recorder. A simplified schematic diagram is depicted in Figure 1.

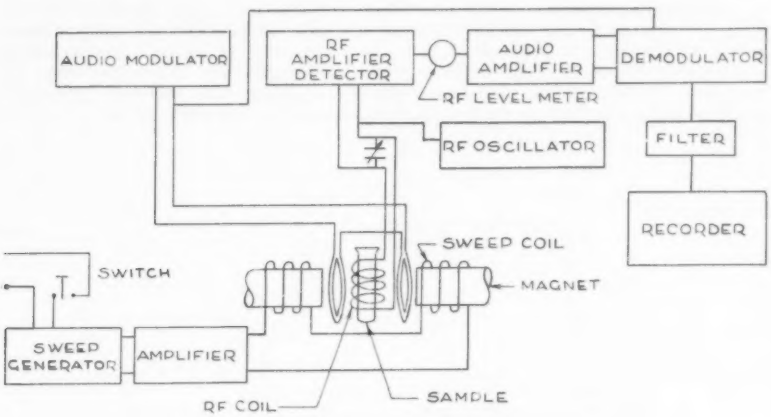

Figure 1. Block diagram of NMR instrument.

Samples of uncooked, lean, smoked, sliced ham were diced and weighed into a glass sample container. The samples were then inserted into a coil located in the center or "eye" of the electromagnet. The oscillator frequency was tuned to the optimum signal response as detected on the oscilloscope and recorder. The integration time and modulation current were also set according to the signal response, after which the initial signal was recorded. The same meat samples were then dried in an air oven for 15 minute intervals at 113° C. Signal response was obtained at each 15-minute interval on the samples at approximately 90° C. and 0° C. The loss in weight of the oven-dried samples were also recorded.

The next study concerned the temperature-signal relationship. Samples of fresh, uncooked frankfurter emulsion were chilled quickly, by means of dry ice, to 7° C. and tested in the instrument. The samples were then warmed and the signal was recorded at 7, 9, 11, 13, 16, 19, and 23° C. The technique was then reversed. Using different samples of the same emulsion, the signal was recorded at 27, 24, 20, 17, 13, 9, 7, 4, and 0° C.

An attempt was made to evaluate the signal response as a function of the fat content in the meat samples. Samples of pork fat and beef fat were ground and blended separately. Water was then added to the samples to give various moisture levels. These moisture contents were measured for each sample by means of a Brabender Semi-Automatic Moisture

Tester (Brabender Corporation, Rochelle Park, New Jersey). NMR signals were obtained for each level.

To determine the fat-protein inter-relationship and its effect on the moisture content and signal response, tests were made on pork muscle, beef muscle, fat pork trim, lean pork trim, fat beef and lean beef trim with the moisture content and NMR signal response being determined as in the previous tests.

In order to understand more about the total water, free water and NMR signal relationships, samples of *semimembranosus* beef muscle were taken from a cutter-canner grade of beef. Total moisture was determined by conventional heat-drying process. Free water was measured by the Grau and Hamm filter paper method *(4)* as modified by Wierbicki and Deatherage *(21)*. pH determinations were made by means of a standard pH meter. These determinations were made on samples of the muscle at post mortem periods of 2, 4, 6, 8, and 24 hours and 2, 5, 7, and 9 days.

The effect of ionic constituents in aqueous solutions was also studied. Samples of raw water having a specific resistance of 2.3×10^3 ohms containing Ca, Mg, Na, SiO_2, R_2O_3, HCO_3, PO_4, SO_4, and Cl ions were compared by the NMR method with samples of deionized water having a specific resistance of 1×10^6 ohms.

RESULTS

Instrument response. It was established that a definite signal could be obtained using meat as a test product and that the signal amplitude increased with increasing moisture content (Figure 2). It appeared that the moisture range of meat is ideally located within the most accurate range of the instrument as adjusted for these experiments. It was further established that the measurement of the peak to peak amplitude (2D-Max) would be more desirable than the line-width measurement of the signal *(9, 14)*.

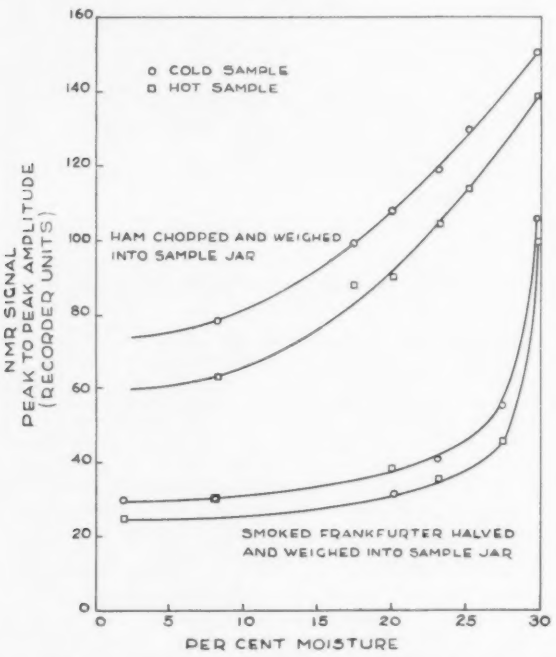

Figure 2. Effect of moisture and temperature on NMR signal response.

Effect of temperature. It was found that different signal amplitudes were obtained on the same sample at 90° C. and 0° C. (Figure 2). Further investigation revealed that on a pre-chilled, uncooked sample, the signal amplitude increased with increasing sample temperature over a range of 7° C. to 23° C. The signals obtained during the warming of the pre-chilled sample resembled the type of signal response obtained upon thawing of ice. However, these results could not be duplicated on chilling a sample from 27° C. to 0° C. (Figure 3). It was concluded that on heating a chilled sample a certain degree of denaturation takes place with the release of free water. The amount of free water available is not re-absorbed by the proteins and therefore the signal variance is less pronounced.

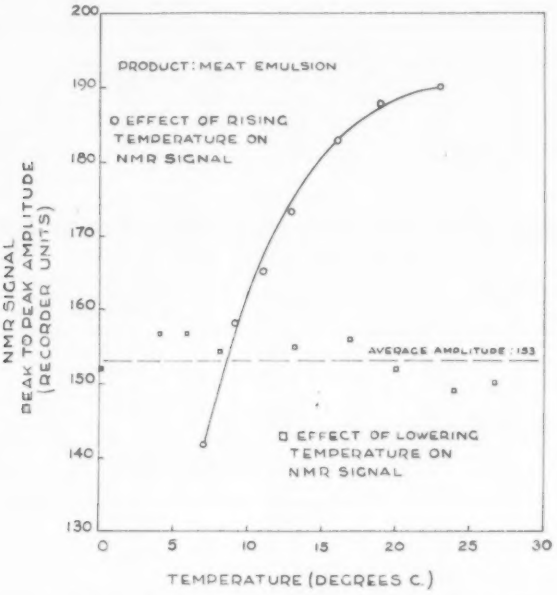

Figure 3. Effect of chilling and heating on NMR signal response.

Since signal amplitudes differed on the same sample at various temperatures, it appears that other factors which are dependent on temperature variation are also concerned with the signal intensity.

Effect of fat. It was found that an NMR signal can be obtained on a sample containing a fat content as high as 92%. It was also observed that the signal appeared intense and narrow in width. The tests indicated the inability of heat-drying methods to give accurate moisture measurement on samples of high fat content. These discrepancies of moisture were observed in both the pork and beef fat samples (Table 1). Although the NMR signal response in beef fat increased with increasing moisture content, it was found that in the case of pork fat an actual decrease in signal intensity occurred with increased moisture content. It was also observed that a sharp signal could be obtained from the samples even though the fat was in a semi-solid state. The tests made on lean pork muscle, and lean beef muscle gave no correlation between the NMR signal response and the calculated increased moisture levels in the samples. It was observed, however, that while the total moisture of the samples, as determined by the heat-drying process varied from 75.4% to 79.8%, the free moisture of the samples varied from 25.7% to 41.1%. The NMR signal response, as measured in recorder units varied from 106.2 units to 205 units with no increase or decrease in signal amplitude apparent which could be employed as a reference curve (Table 2). It was concluded that the signals are not

TABLE 1

Effect of varying moisture levels in pork fat and beef fat on NMR signal

Moisture (%)		Fat (%) by extraction	Sample Temp. degrees C.	NMR Signal recorder units
Calculated	Brabender			
TEST A. PORK FAT				
——	8.0	91.88	20	105.6
12.4	14.0	85.88	20	119.3
16.4	18.6	81.10	20	84.1
23.3	24.0	75.85	20	90.7
TEST B. BEEF FAT				
——	4.4	92.39	20	153.4
8.9	6.8	91.83	20	153.7
13.1	9.8	92.18	20	160.1
20.3	15.0	88.26	20	171.6

TABLE 2

Effect of varying moisture levels in lean beef and lean pork muscle on NMR signal

Moisture (%)		Fat (%) by extraction	Protein (%) by difference	NMR Signal recorder units
Calculated	Brabender			
TEST 1. LEAN PORK MUSCLE				
——	76.2	1.26	22.54	159.2
77.4	77.4	1.20	21.40	154
78.4	78.4	1.15	20.45	205
80.1	79.8	1.07	19.13	108
FREE MOISTURE 32.4% *				
TEST 2. LEAN PORK MUSCLE				
——	75.8	2.46	21.74	113.9
76.8	76.8	2.38	20.82	136.1
77.9	77.6	2.25	20.15	125.7
79.6	79.2	2.17	18.63	129.2
FREE MOISTURE 38.3% *				
TEST 3. LEAN BEEF MUSCLE				
——	75.4	2.50	22.1	176.2
76.6	76.8	2.56	20.64	107.5
77.5	77.3	2.59	20.11	174.2
79.4	79.0	2.62	18.38	106.2
FREE MOISTURE 25.7% *				
TEST 4. LEAN BEEF MUSCLE				
——	75.4	1.93	22.67	137.1
76.6	76.8	2.13	21.07	137.5
77.5	78.0	2.27	19.73	128.1
79.4	79.2	2.28	18.52	128.7
FREE MOISTURE 41.1% *				

* Measured by the method of Wierbicki and Deatherage (21).

dependent on moisture levels, but that temperature, fat content, protein content and the association of water to the protein or fat in the sample most likely contribute to the instrument signal response.

Effect of ionic materials. It was found that there was a definite increase in the amplitude of the signal in a water solution containing ionized materials. While insufficient tests were made, it appears that the effects of added mineral constituents such as salt,

nitrate, nitrite, phosphate, etc., would have a definite influence on the signal since these additives change the ratio of free water to the protein bound water in the sample *(20, 21)*.

Effect of free moisture shift in beef muscle. It was found that the free moisture content of the *semimembranosus* beef muscle increased during the first 48 hours post mortem. Little increase was observed in the NMR signal during the first 6 hours. The per cent total moisture remained fairly constant for the entire 9-day test period. It was found that there was an increase in signal response from 8 to 24 hours post mortem and then the signal decreased uniformly in intensity over the balance of the 9-day test period (Figure 4). This followed closely the corresponding decrease in per cent free moisture content of the muscle for the same period of time (Figure 5). It was observed

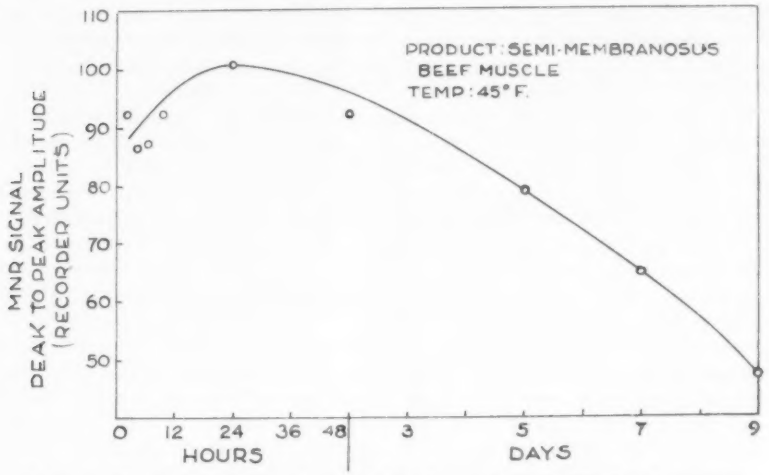

Figure 4. Effect of chilling and aging of beef muscle on NMR signal response.

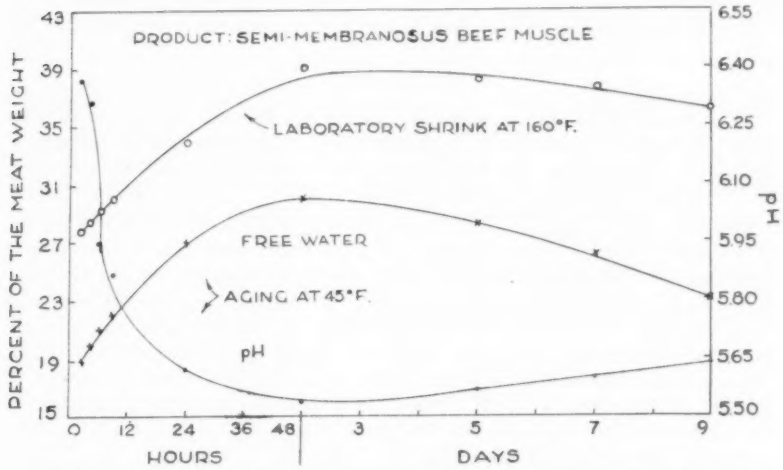

Figure 5. Effect of chilling and aging on pH and free water and laboratory shrink at 160° F.

that the maximum signal was obtained at 24 hours while the laboratory methods show the minimum hydration of the beef tissue at 48 hours. This similarity is observed on curves which are plotted to represent the increase and decrease in signal amplitude with the increase and decrease of free water in fresh and cooked meat. These curves also follow the measurable shrink *(21)* that is apparent in chilling and aging of the beef round.

DISCUSSION

Although the mechanics of protein hydration are not fully understood, it was demonstrated that the free water of lean beef samples, as measured by the Grau and Hamm filter paper method as modified by Wierbicki and Deatherage *(21)*, was 30% to 50% of the total moisture as measured by the heat-drying process (Table 2). It is generally believed that the polar side chains of amino-acid residues of protein provide much of the attraction of the absorbed water molecule. It is further to be expected that the different polar groups of a protein would attract water molecules with different avidity. Pauling *(8)* has made a comparison of the number of water molecules held by proteins in initial absorption and the number of polar groups in the proteins and found a significant water absorption in the first layer between different material. He has also found an agreement between the number of molecules initially absorbed and the number of polar groups in the protein, thus confirming the results by Bull *(3)* and Shaw *(15)*. It has been demonstrated that the highly polar molecules of water can be attracted to muscle proteins by ionization of acidic and basic groups, as well as the polar non-ionic groups. Thus, in a meat sample we can expect to find a protein-water relationship which has been defined as bound or sorbed water as opposed to water which is disassociated with proteins or free water *(19, 21)*. The exact amount of free water contributing to the NMR signal must be understood before this method can be employed as an analytical tool in the meat processing industry. This measurement of free water is of importance in NMR spectroscopy as it has been demonstrated that liquids containing hydrogen compounds have different NMR absorption lines than those of solids which contain hydrogen *(17)*.

SUMMARY

It was found that the instrument (Figure 1) gave a definite measurable signal. It was observed that temperature plays a definite role in the signal intensity. It was found that different types of meat and meat products gave rise to different signals. Although tests were limited, it appeared that mineral constituents in aqueous solutions had a definite effect on the NMR signal.

In an attempt to correlate signal response with conventional heat-drying methods, the results were erratic. This was due, especially in fat samples, to the inability of the heat-drying methods to differentiate between free and total moisture. When the method of Grau and Hamm, as modified by Wierbicki and Deatherage *(21)* was employed, significant results were obtained and there was correlation of these results with the NMR signal response. It was also found that the NMR method detected changes taking place in meat during post mortem periods, before these changes were detected by the conventional laboratory methods (Figures 4 and 5).

At the conclusion of our work, it appeared that the instrument under investigation was not applicable as an analytical tool for the control laboratory.

However, an instrument of greater scope and resolution might play a significant role in revealing important physical changes and inter-relationships taking place in meat structure.

Acknowledgment

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PHYSIOLOGICAL PROPERTIES OF GROUP D STREPTOCOCCI ISOLATED FROM PLANTS[a]

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(Manuscript received May 31, 1958)

With the information then available, Sherman *(13)* condensed those streptococci possessing the properties termed the Sherman characteristics into three species. One of these, *Streptococcus faecalis,* is commonly accepted as an indicator of pollution. Subsequent studies by Barnes *(2)*, Barnes and Ingram *(3)*, Ingram and Barnes *(6)*, and Cooper and Ramadan *(4)* indicate that within this species a distinction may be made, with *S. faecalis* designating the organism of human origin and *S. faecium* designating that of porcine or non-human origin.

Group D streptococci isolated from plant parts as flowers, leaves, shoots, and ovules, and from the atmosphere of a freezing-processing establishment by Mundt, Johnson, and Khatchikian *(11)* were divisible into two groups, those similar to *S. faecalis* and a larger group more closely related to *S. faecium*. It is not known whether these isolates represent a third group. This study was undertaken to ascertain the physiological characteristics according to recently suggested as well as older criteria.

EXPERIMENTAL

Of the 135 isolates included in this study, 116 were taken from plants and 19 from the atmosphere of a freezing-processing establishment. All isolates were Gram positive, elongated to spherical cells occurring in pairs and in short chains, which reacted with commercial group D serum. Five known cultures of *S. faecalis,* obtained from Dr. C. F. Niven, Jr., were studied in parallel.

The basal medium consisted of mixed peptone (BBL polypeptone), 1.5%; beef extract, 0.45%; NaCl, 0.75%; and glucose, 0.75%. Agar was added as needed. The following sugars replaced glucose at 0.5% concentration, with the addition of brom cresol purple as the indicator: alpha-methyl-glucoside, arabinose, glycerol, inulin, mannitol, melibiose, melizitose, sorbitol, raffinose, and sucrose. Fermentation of glycerol was determined anaerobically. Citrate was used as the sodium salt at 1.0% concentration with brom thymol blue as the indicator. Hydrolysis of aesculin was determined according to the method of Diernhofer as given by Little and Plastridge *(10)*.

Tubes of litmus milk and of milk containing 0.1% methylene blue (86% dye content) were used to determine reducing ability and also ability to digest casein. Reducing ability was also observed on agar containing 2, 3, 5-triphenyltetrazolium-HCl, prepared as specified by Barnes *(2)*. Reduction of janus green as suggested by Ayers, Johnson, and Mudge *(1)* imparted no information beyond that obtained by other methods, and its use was discontinued. The method of Cooper and Ramadan *(4)* for determining reducing properties with this dye was not attempted.

Chemical tolerance was determined by the addition of crystal violet to a final concentration of 1:400,000 to sterile agar, and potassium tellurite to agar at pH 7.4 to yield a final concentration of 1:2,500.

Diacetyl was detected by the addition of alpha naphthol and KOH to broth cultures,

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[b] Approved for publication by the Director, Agricultural Experiment Station.

[a] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, May.

and ammonia by the addition of Nessler's reagent to drops of 4% peptone and of arginine broths.

Penicillin was added to the basal agar at the rate of 0.65 units per ml. as suggested by Winter and Sandholzer *(15)*.

Human blood from a blood bank, citrated bovine blood, and washed ovine erythrocytes were added to prepared blood agar base for the determination of hemolysis.

The need for folic acid was determined on folic acid assay medium containing agar.

RESULTS AND DISCUSSION

As is shown in Table 1, the plant isolates conform to the criteria established by Sherman, Mauer, and Stark *(14)* for *S. faecalis*, and thus all are closely related to this organism. A few cultures failed to reduce methylene blue, and the majority exhibited delayed reduction of this compound. Three isolates failed to produce ammonia; this failure was consistent with both media used. All isolates grew well in broth containing ethyl violet, a selective agent for the enterococci, the use of which was suggested for this reason by Litsky, Mallman, and Fifield *(9)*.

TABLE 1

Characteristics of Stereptococcus faecalis and those of 135 isolates from plants

Characteristic	*Streptococcus faecalis*	Isolates
Growth at 10° C. and 45° C.	+	+
Growth in 6.5% NaCl	+	+
Growth at pH 9.6	+	+
Reduction of methylene blue	+	+ (rarely −)
Fermentation of sucrose	+	+
Hydrolysis of aesculin	+	+
Production of ammonia	+	+ (3 −)
Final pH less than 5.0	+	+
Growth in ethyl violet	+	+

Primary differentiating criteria which divide the plant isolates into 2 distinct groups are shown in Table 2. Ten of the 46 *S. faecalis*-like strains failed to grow on agar containing potassium tellurite, and 18 failed to ferment melizitose. Otherwise, they conformed to the description of *S. faecalis*. In general, the 89 isolates placed into the *S. faecium*-like group conformed to descriptions by Barnes *(2)* and Lake, Deibel, and Niven, *(8)*. They did not grow on agar containing potassium tellurite, and reduction of the tetrazolium

TABLE 2

Major characteristics dividing 135 isolates into streptococcus faecalis-like and S. faecium-like groups

Characteristic	*Streptococcus faecalis-like*	*Streptococcus faecium-like*
Growth on tellurite	Strong (10 —)	Weak or none
Reduction of tetrazolium	Strong	Weak or none
Reduction of Litmus	Strong	Usually weak
Fermentation of glycerol	+	− (7 +)
Fermentation of mellibiose	−	+ (2 −)
Fermentation of melizitose	+ (18 −)	− (1 +)
Fermentation of arabinose	−	+ (11 −)
Fermentation of sorbitol	+	− (8 +)

salt was either negative or less intense than was the reduction by the *S. faecalis*-like organisms. A small number deviated from the fermentation pattern, and the numbers of cultures which deviate are included in the table. Barnes *(2)* has stated that sorbitol is fermented by some strains of *S. faecium*.

Responses of the isolates to additional criteria are shown in Table 3, and are compared with the responses of known *S. faecalis* and *S. faecium* (the latter as taken from the literature). All *S. faecalis*-like isolates corresponded with the known cultures in their response to citrate, inulin, mannitol, raffinose, diacetyl production, and heat. It has been reported that *S. faecalis* ferments alpha-methyl-glucoside; neither the isolates nor the known cultures fermented this compound in this study. Thirty-two isolates required folic acid for growth. None brought about a strong reduction of methylene blue in milk in less than 2 days, and all isolates produced a "greening" reaction on agar containing ovine erythrocytes.

TABLE 3

Reactions of isolates and those of described species

Property	*Streptococcus faecalis*		*Streptococcus faecium*	
	Description	Related Isolates	Description	Related Isolates
Fermentations:				
Alpha-methyl-glucoside	+ [1]	−	−	Var.
Citrate	+	+	−	Var.
Inulin	−	−	[2]	Var.
Mannitol	+	+	[2]	Var.
Raffinose	−	−	[2]	Var.
Diacetyl production	+	+	[2]	Var.
Folic acid required	−	Var.	+	Var.
Greening, sheep cells	−	+	+	+
Survive, 60 C., 30 minutes	+	+	−	Var.
Reduction of methylene blue	Rapid	Slow	[2]	Slow

[1] Known cultures were negative.
[2] Property is not known.

In contrast, the *S. faecium-like* group was variable to all criteria except those of greening of ovine erythrocytes and of reduction of methylene blue. The majority of the group also fermented citrate, survived heating to 60° C. for 30 minutes, many fermented alpha-methyl-glucoside, and 16 cultures did not require folic acid for growth. The 15 cultures not fermenting mannitol also failed to ferment inulin. A number fermented raffinose, whereas neither known *S. faecalis* nor the related isolates fermented this sugar. The deviations within the *S. faecium*-like group are classified and summarized in Table 4.

Reactions in milk, and responses to crystal violet and to penicillin are shown in Table 5. All *S. faecalis*-like isolates and 17 of the *S. faecium*-like strains produced a curd in litmus milk within 2 days. Nineteen of the latter produced no curd on the ninth day, when incubation was discontinued. Thirty-three *S. faecalis*-like strains digested casein within 9 days, all grew on agar containing penicillin, and 19 grew on agar containing crytsal violet. None of the *S. faecium*-like isolates digested casein nor grew on agar containing crystal violet, but 29 of those tested tolerated penicillin. Thus, it appears that penicillin is not specific for members of the *S. faecalis*-like group, while crystal violet will not enable all the strains of this group to grow.

TABLE 4

Physiological deviations of the S. faecium-like isolates from those of the described species

Characteristic	Ferment citrate	Citrate not fermented
None (except citrate)	15	12
Fermentation of AMG [1]	20	1
Fermentation of sorbitol	3	1
Fermentation of AMG and sorbitol	4	0
Fermentation of AMG and glycerol	6 [2]	0
Fermentation of glycerol	0	1
Arabinose not fermented	2	5
Melibiose not fermented	2	0
Folic acid not required	6	1
Folic acid not required, AMG fermented	4	0
Folic acid not required, arabinose not fermented	2	2
Folic acid not required, melibiose not fermented	1	0

[1] AMG — alpha-methyl-glucoside
[2] Two cultures also fermented both citrate and sorbitol.

TABLE 5

Curd reactions in litmus milk, and on agars containing crystal violet or penicillin

Substrate	*Streptococcus faecalis*-like	*Streptococcus faecium*-like
Curd formation	1-2 days	17: 1-2 days 53: 3-9 days 19: no curd, 9 days
Digestion of casein	33: 2-9 days 13: none at 9 days	none
Grew on crystal violet agar	19	none
Grew on penicillin agar	46	29 strong 14 moderate 16 none 30 not tested

No culture included in this study digested starch or hemolyzed human red blood cells. Hemolysis of bovine cells was variable and frequently it was delayed.

Although there is some evidence for the existence of plant epiphytic streptococci related to the enterococci, at this time is must be considered presumptive. Within the group of the *S. faecalis*-like isolates, only 2 properties, hemolysis of ovine erythrocytes and generally slow reduction of methylene blue, consistently distinguish them from *S. faecalis* of human origin. Only 6 of the 46 cultures conformed exactly in their fermentative pattern to *S. faecalis* as described by Lake *et al.* (8). A total of 72% of this group were caseolytic, a figure very similar to the 73% reported by Kaplan and Appleman *(7)*. Sherman *(12)* considered as a point against accidental occurrence of the group on plants the fact that no hemolytic types had been isolated from plant sources. Since the isolates under study hemolyzed ovine erythrocytes and a number hydrolyzed bovine cells, it must be assumed that he used blood of another source. Guthof *(5)* has reported that enterococci of the liquefying type are nearly non-existent in the intestinal tract of normal, healthy humans. Upon this point, the authors have no further knowledge.

Twelve of the 89 *S. faecium*-like group conformed exactly to the description given by Lake *et al. (8)*. Although the members of this group are set apart quite easily from the *S. faecalis*-like group, no criteria have been discovered which would set apart the plant isolates from *S. faecium* as this organism has been described. The strong deviations in the fermentative patterns are suggestive of differences, but the patterns are neither uniform nor correlative, and therefore appear to be of little taxonomic value.

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SUMMARY AND CONCLUSIONS

The 135 isolates taken from plants and from the atmosphere of a freezing-processing establishment have but two properties in common which are not shared by *S. faecalis* of human origin: the hemolysis on agar containing ovine erythrocytes and the generally slow reduction of methylene blue. With these exceptions, 6 of the 46 cultures placed into the *S. faecalis*-like group conformed exactly to the expanded description of *S. faecalis;* the remainder deviated in one or more physiologic properties, primarily in the need for folic acid and the failure of 18 cultures to ferment melizitose. Seventy-two per cent of the isolates in this group were caseolytic.

All the *S. faecium*-like cultures conform to the basic pattern of properties established for *S. faecium*. However, 77 cultures deviate from the expanded description by the resistance to heat on the part of many, and in the ability of numbers of cultures to ferment sugars to which *S. faecium* is negative.

The deviations in fermentations and in tolerance to crystal violet and penicillin were not consistent, and the deviations were not correlative with each other or with other properties. Therefore, they are not considered reliable from a taxonomic point of view.

It is concluded that although there is some cumulative evidence for the existence of an independent plant flora, no common property has been uncovered thus far to confirm such existence.

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ACTIVATED CARBONS AS ODOR SCAVENGERS FOR RADIATION-STERILIZED BEEF [a, b]

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A major obstacle to the use of ionizing radiation in food preservation has been the production of objectionable qualities in the foods thus processed. In meats, and particularly in beef, off-odors and flavors have been the most prominent of the changes produced.

The use of activated carbons as in-package odor scavengers to remove irradiation-produced odors was considered because of proven utility with a variety of products *(3)* and also because the conditions under which radiation-sterilized meats will be used (i. e., storage at non-refrigerated temperatures) are such as to enhance any "scavenging" properties which might be exhibited by an adsorbent material. The complex nature of irradiation odors suggested that adsorbent materials other than charcoal also might prove useful. In the study outlined below, three separate experiments were performed.

Experiment No. 1. Heat enzyme-inactivated ground beef (internal temperature 175° F.) was vacuum- or nitrogen-packed into cans along with packets of the adsorbent material. Activated carbons used were: Pittsburgh Coke & Chemical Company, Type BPL (12-30 mesh), Type OL (20-50 mesh); West Virginia Pulp and Paper Company, Type Nuchar C (unground); the Barnebey-Cheney Company, Type T-33 Pac, and Ag^+, Cu^+ impregnated carbon. Other adsorbent materials used were: Porocel Corporation of International Minerals, bauxite desiccant (6-14 mesh); the Floridin Company, Florex B (fuller's earth), Calcined Florex B (fuller's earth), Florite (activated bauxite), Florosil (magnesium silicate). The packets were made by heat-sealing the adsorbent material within a bag made from heat-sealable non-woven fabric. All cans were irradiated (dose of 4.6 megarads), and stored at 72° F. for periods up to 6 months. Odor evaluations were performed at 2-month intervals by a 4-6 member informal laboratory panel. Results are shown in Table 1.

[a] Paper No. *898* in the series of papers approved for publication.
[b] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists held in Chicago, Illinois, May 27, 1958.

TABLE 1

The effect of different adsorbent materials in removing "irradiation odor" from heat-enzyme-inactivated, 4.6 megarad irradiated ground beef during storage at 72° F.

Adsorbent	Odor intensity scale (1-9)				
	Storage (in months)				
	0	1	2	4	6
No adsorbent (vacuum pack)	9	9	9	9	8
Activated carbons (Ave.) (vacuum pack)	6	4	3.2	3.3	3.0
Activated carbons (Ave.) (nitrogen pack)	*	*	*	*	4.1
Non-carbon materials	9	9	9	**	**

* Nitrogen-packed samples only evaluated after 6 months storage.
** Odor evaluation on non-carbon adsorbents discontinued after 2 months.

Little difference was noted between the various activated carbon samples, and the values for these groups were averaged. A similar lack of differentiation and a general increase in odor intensity were observed with the group of non-carbon adsorbent materials. The significant variables for decreasing the irradiation odor intensity in heat-enzyme-inactivated ground beef were shown to be the use of activated carbon and storage [c]. Activated carbon with vacuum-packing exhibited a slightly greater efficiency of adsorption than that with nitrogen-packing; this was probably due to a higher rate of diffusion of the odor molecules from the meat to the adsorbent.

The increased intensity of irradiation odor which occurred during storage of samples packed with adsorbent materials other than carbon was of interest. This finding may be related to the post-irradiation reaction chemistry of irradiation odors and flavors and further investigations in this area may be enlightening. The result noted could be due solely to an increased content of oxygen in the sample cans because of the larger surface area of adsorbent materials. A similar increase in intensity occurring in samples with activated carbon could go unnoticed because of an adsorption of the oxidation products. Of the many factors which might be involved, the possibility of a catalysis of the volatile irradiation odor compounds on a metallic oxide surface seems worthy of mention, because metallic oxides are common to all five of the non-carbon adsorbent materials in question.

Experiment No. 2. Samples were vacuum-packed, irradiated, stored, and evaluated as in the previous experiment except (a) the adsorbent materials were various activated carbon materials supplied by the Barnebey-Cheney Company (b) both raw and heat-enzyme-inactivated samples (internal temperature 165° F.) were made up and (c) an additional odor evaluation was performed on the samples after cooking. Figure 1 illustrates the averaged odor-evaluation results.

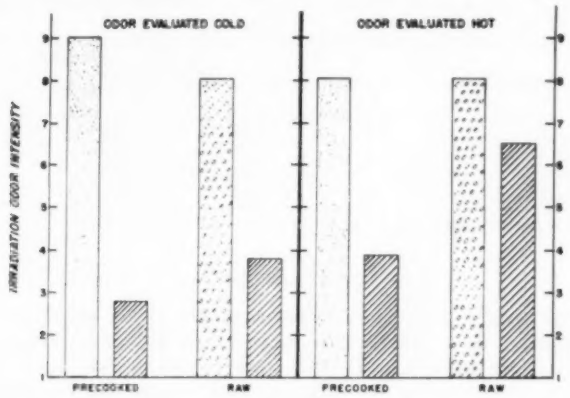

Figure 1. The effectiveness of activated carbon ▭ for removing "irradiated odors" from irradiated (4.6 megarads) cooked and raw ground beef during 72° F. storage for 2 to 4 months. Evaluations made on room temperature samples, and on samples heated under a low-fire gas broiler.

The slight increase in irradiation intensity noted in the carbon-packed pre-irradiation cooked ground beef upon heating is consistent with respect to the diffusion/temperature equilibrium expected where most of the volatile compounds have been removed. The large increase of odor intensity on heating the carbon-packed raw ground beef, however, suggests

[c] Two additional sets of cans were packed with raw meat and these cans were given the normal thermal-processing (45 minutes at 250° F. and 5 lbs. pressure). No effect of odor adsorption by activated carbon or non-carbon materials was noted in any of these samples after 72° F. storage periods of 2 and 6 months.

that the relatively wet mass of raw beef material prevented effective diffusion of such compounds from the interior of the sample to the surface, or that the volatile chemical compounds constituting the irradiation odor in cooked beef exist as non-volatile heat-labile precursors in the raw meat. The mechanism of the occurrence is mostly of academic interest at this time, however, since the storage of radiation-sterilized raw meats has been precluded until the enzyme activity can be controlled by non-thermal means *(2)*.

Experiment No. 3. Beef "rib-eye" steaks were randomly selected from three pairs of matched ribs, and were heat-enzyme-inactivated by heating on a grill in a steam retort at atmospheric pressure to an internal temperature of 165° F. (the drip was discarded). Carbons used were selected from those supplied by the Barneby-Cheney Company for Experiment No. 2 as follows:

No. 1. Material suitable for adsorption of large, high molecular weight compounds.

No. 2. Deodorizing carbon of extremely high activity.

No. 3. Blend of Nos. 1 and 2 (1:1).

Steaks were vacuum-packed along with the packets of carbon in No. 2½ beaded tinplate interior-enameled cans, irradiated (dose of 4.6 megarads), and stored at 72° F.. At 2, 4, and 6 months, samples were evaluated for intensity of irradiation odor/flavor using a formal panel. Mean intensity ratings based on 32 judgments for each sample were obtained. Flavor evaluation results are shown in Table 2.

TABLE 2

The effect of using activated carbons to decrease the intensity of "irradiation odor/flavor" during 72° F. storage of heat-enzyme-inactivated, 4.6 megarads irradiated beef rib-eye steaks

Activated carbon	Intensity scale (1-9)		
	Storage (in months)		
	2	4	6
Type I	3.10	3.06*	3.78*
Type II	4.13	2.59*	2.82*
Type I: Type II (1:1)	3.88	2.91*	3.00*
No carbon	3.35	4.63	5.84

* Significant (activated carbon vs. no carbon) at a confidence level of 1%.

CONCLUSION

Since the results obtained in Experiment No. 3 are based on an actual flavor evaluation by a large panel rather than on odor alone, they perhaps best indicate the potential of activated carbons for use in removing irradiation odor/flavors in radiation-sterilized meats. Such results also indicate that activated carbons should be a valuable research tool for identifying the nature of objectionable flavors produced in irradiation-sterilized meats, either by providing a method of isolating and concentrating the volatile compounds or by providing substrates in which quantitative differences in content of volatile compounds can be made.

In the interpretation of this study for a wider application of the use of activated carbons in radiation-sterilized foods, it is clear that considerably more information must be acquired. For example, the data indicate only that activated carbons are effective in reducing irradiation odor/flavor intensity. That lessening of this property should result in increased acceptance is, of course, inherent in the purpose of the experiment, but the specific evaluation of acceptability for such products is yet to be accomplished. This study also did not evaluate the extent to which adsorbents might remove

desirable natural odors and flavors during storage. This could become particularly important in a product such as chicken where the characteristic cooked flavor is of a volatile nature *(1)*.

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THE RELATIONSHIP BETWEEN PANEL SCORES AND CERTAIN CHEMICAL COMPONENTS IN PRECOOKED IRRADIATED MEATS [a, b, c]

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The most critical problem in connection with the acceptance of irradiated meats is the development of a characteristic flavor, commonly referred to as "irradiation flavor". The magnitude and occurrence of the off-flavor problem differs with the kind of meat *(10)* and the level of radiation *(7)*. Although a number of chemical components have been shown to increase markedly on radiation *(2, 3, 7)*, no attempt has been made to correlate panel scores and the amounts of various chemical components present.

Consequently, this cooperative study was initiated to investigate the relationships between the level of certain chemical components in irradiated meats and panel scores.

Experimental Procedure

Preparation, Irradiation and Storage

Beef. The *Longissimus dorsi* muscles were removed from 3 pairs of U. S. Choice beef ribs, which originated from cattle fed and managed similarly. Paired right and left roasts were cut to fit into No. 2 cans, inserted into polymylar bags, placed in the cans and roasted to an internal temperature of 71° C. in an electric oven at 149° C. The bags were heat sealed following roasting; the cans were closed under 28 inches of vacuum and cooled to 2° C. All cans were coded and labeled in order to identify all roasts, so that one roast from each pair could be presented to the panel and the pair-mate could be used for chemical analysis. Paired samples representing each pair of ribs were used as unirradiated controls while the remainder was divided into 3 groups to be radiated. Each group was composed of the paired roasts from the same carcass.

Two groups were radiated to a level of 2.79 megarads with a gamma source, after which one of the groups was stored at approximately 24° C. while the other was held at 2° C. The remainder were radiated with 1.86 megarads and stored at 2° C. Storage was for 0, 1, 3, and 6 months for both temperatures of storage and for both levels of irradiation.

Veal: Eight pairs of veal shoulder clods were cut into roasts and handled the same as the beef, except for the fact that they were randomly allotted into groups, roasted to an internal temperature of 77° C., and stored at 38° C. in addition to the temperatures used for beef. Thus, storage was for 0, 1, 3 and 6 months at 2° C., 24° C. and 38° C. All the roasts radiated at 1.86 megarads were stored at 2° C. and those irradiated at 2.79 megarads were divided among all 3 temperatures.

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[a] Journal Article *2274* Michigan Agricultural Experiment Station, East Lansing.

[b] Journal Paper No. *163*, American Meat Institute Foundation.

[c] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number *932* in the series of papers approved for publication.

Pork: Two pairs of boneless pork loins were cut into chops ¾ in. in thickness, and deep-fat fried in lard held at approximately 138° C. until the chops reached an internal temperature of 82° C. Three chops were placed in each polymylar bag, otherwise the sealing, irradiation and storage procedure was the same as that used for veal.

Panel Ratings

Prior to submitting the samples to the taste panel, several small cores were removed from the various sections of each can and subjected to bacteriological and toxicity tests. After reasonable freedom from toxins and harmful bacteria was assured, which usually required about 3 days, the samples were released for panel testing. The opened cans were then heated for 50 minutes at 149° C. in an electric oven prior to presentation to the panel.

A consumer type panel composed of 18 judges was used throughout the study. Although there was considerable variation in the experience of panel members, most of them had participated in previous tests on radiated meats. No attempt was made to obtain the same judges for all panels, but most members served on more than one panel in the course of the study. The samples were rated for over-all acceptability on the 9-point hedonic scale *(11, 12)* with the higher score indicating that the samples were more acceptable. Average panel ratings were used in the correlation analysis with all chemical components.

Chemical Analysis

Preparation: The samples were removed from the cans and polymylar bags and ground three times through a food chopper fitted with a plate having 1/16 inch openings. The samples were thoroughly mixed after grinding each time and aliquot samples were used in all subsequent chemical determinations.

Routine Analysis: Moisture, fat and protein were analyzed by A.O. A.C. methods *(1)*. Determination of pH was made on a slurry of 10 g. of meat plus 25 ml. of distilled water.

Other chemical components. Hydrogen sulfide was determined by the method of Marbach and Doty *(9)* and methyl mercaptan as described by Sliwinski and Doty *(13)*. Acid-soluble carbonyl compounds were estimated by a modification of the method of Lappin and Clark *(8)* as described by Batzer, Sribney, Doty and Schweigert *(3)*. Reduced glutathione was determined by the method of Grunert and Phillips *(6)*, whereas total glutathione was measured on the same solution after electrolytic reduction by the procedure of Dohan and Woodward *(5)*. Glycogen determinations were made by the method of Carrol, Longley and Roe *(4)*.

Statistical Analysis

Simple correlation coefficients were calculated for all chemical components and panel score according to the method described by Snedecor *(14)*. Multilinear correlation coefficients *(14)* were computed for panel score with various combinations of hydrogen sulfide, methyl mercaptan and carbonyl groups, which were the single chemical components appearing to contribute most to irradiation flavor.

RESULTS AND DISCUSSION

Chemical Analyses and Taste Panel Evaluations

Results from chemical analyses and taste panel evaluation (Table 1) show that irradiated precooked meats contained more hydrogen sulfide, methyl mercaptan and acid-salt soluble carbonyls than unirradiated cooked meats. The effect of radiation on the glutathione content of cooked meats was not as pronounced or as consistent as has been reported for raw beef *(2)*. Panel scores were markedly lower for the irradiated than for the unirradiated meat. It is obvious (Table 1) that the acceptability of pork was less adversely affected by radiation than was true for veal, and particularly, for beef. The effects of storage and level of radiation will be discussed in a subsequent publication.

TABLE 1

Ranges in panel score and chemical composition of cooked beef, pork and veal radiated at different levels prior to storage [1]

Cooking Treatment	Level of Radiation	Panel Score	Hydrogen Sulfide μ/g.	Methyl Mercaptan μ/g.	Total Glutathione mg./100 g.	Reduced Glutathione mg./100 g.	Carbonyl Compounds $\times 10^5$ M g.	pH	Glycogen mg./g.	Moisture %	Fat %	Protein %
						Beef [2]						
Roasted	0	6.33-7.33	0.55-0.76	0.20-0.23	——	10.87-17.75	2.53-3.36	5.80-6.30	0.00-1.07	63.8-68.2	8.6-12.6	20.8-24.9
Roasted	1.86×10^6 rads	4.22	1.04	0.45	——	13.13	7.02	5.80	0.00	66.2	9.1	24.9
Roasted	2.79×10^6 rads	3.28-3.72	1.02-1.20	0.48-0.56	——	8.50-12.00	8.63-9.09	5.80-6.28	0.00-0.94	63.8-64.3	10.3-12.2	23.8-25.7
						Pork						
Fresh Frozen	0	——	0.00	0.13	28.25	8.00	0.92	5.90	0.00	62.4	17.6	18.7
Deep fat fried	0	6.61-6.89	0.74-1.35	0.13-0.20	14.50-17.75	4.13-6.25	1.56-2.19	6.30-6.35	0.00-0.00	40.8-52.7	16.7-37.3	21.6-31.2
Deep fat fried	1.86×10^6 rads	6.89	2.04	0.30	13.75	6.13	7.42	6.20	0.00-0.00	49.5	22.4	27.5
Deep fat fried	2.79×10^6 rads	4.94-5.55	1.19-2.86	0.30-0.57	12.50-14.36	4.00-5.38	9.44-11.61	6.20-6.20	0.00-0.00	47.7-49.0	20.3-27.2	25.2-30.7
						Veal						
Fresh Frozen	0	——	0.10	0.40	17.50	4.25	2.99	5.55	0.00-0.00	75.5	2.6	21.5
Roasted	0	5.78-7.05	0.56-1.12	0.27-0.55	9.50-12.00	0.00-3.75	2.76-3.82	6.00-6.10	0.00	66.4-73.6	1.1-8.1	24.5-27.0
Roasted	1.86×10^6 rads	5.17	1.95	0.53	16.25	3.50	7.13	6.30	0.00	70.7	2.3	26.0
Roasted	2.79×10^6 rads	3.72-5.05	2.27-2.46	0.66-1.30	8.00-10.75	1.50-3.25	9.13-10.12	6.30-6.38	0.00-0.51	72.5-75.1	1.8-2.6	21.2-25.6

[1] Where only one value is given, a range is not available as only one sample was analyzed. All other ranges include 2 to 4 samples.
[2] Blank values for total glutathione under beef indicate that this determination was not made.

Simple Correlation Coefficients

A summary of the simple correlation coefficients is given in Table 2. The coefficients between panel score and percent fat are negative, but are not statistically significant. Apparently fat level has little if any influence on irradiation flavor. Similarly, carbonyls are negatively associated with panel scores, although the relationships are much higher than those for fat. It appears probable that the carbonyls associated with irradiation flavor are break-down products of compounds other than fat. This theory is not only supported by the low relationships between percent fat and panel score, but also by the fact that the correlations between percent fat and carbonyls are negligible ($r = .08$ for beef, .05 for pork and $-.04$ for veal). Futhermore, the magnitude of the association of carbonyls and panel scores is consistently as high or higher than that of any other single chemical component for all 3 types of meat. Thus, if a single chemical component were to be selected as an indicator of panel score for irradiated meats, carbonyls appear to be most promising, although they probably do not contribute directly to the typical odor and flavor of irradiated meat.

TABLE 2

Simple correlation coefficients between panel scores and amount of chemical components in irradiated beef, pork and veal

Chemical Components	Beef [1]	Pork [2]	Veal [2]
Percent fat	−.19	−.18	−.04
Percent moisture	.38	.16	−.08
Percent protein	−.24	.12	.43*
pH	−.07	.28	——[4]
Hydrogen sulfide-gamma/gm.	−.46*	−.25	−.50*
Glycogen-mgs./gm.[3]	.15	.00	——[4]
Methyl mercaptan-gamma/gm.	−.76**	−.54*	−.22
Total glutathione-mgs./100 gms.	——[4]	+.08	−.12
Reduced glutathione-mgs./100 gms.	.48*	−.02	−.31
Carbonyls− × 10^{-5}M/gm.	−.74**	−.57**	−.49*

[1] n = 17 [2] n = 20
[3] Only 4 samples contained measurable amounts of glycogen and all were beef.
[4] Values were not available for calculating correlations.
* Signifiant at 5% level. ** Signifiant at 1% level.

Hydrogen sulfide and panel score were also negatively correlated, with relationships ranging from −.25 for pork to −.50 for veal. The coefficients of determination show variation in hydrogen sulfide content accounts for only 21, 6 and 25 percent of the variation in panel score for beef, pork and veal. However, the consistency of predictability for the three types of meat indicates that hydrogen sulfide contributed more to irradiation flavor than any of the chemical components studied with the exception of carbonyls and methyl mercaptan.

The level of methyl mercaptan was also one of the more promising chemical components for indicating panel ratings. Correlation coefficients were −.76, −.54 and −.22 for beef, pork and veal, respectively. Thus, the level of methyl mercaptan accounted for 58, 29 and 5% of the variation in panel score for beef, pork and veal, respectively. Since methyl mercaptan

has been identified as one of the odorous compounds which increases on irradiation *(2, 7, 11)*, the rather good predictive value was to be expected.

Moisture and protein content and level of reduced glutathione vary both in direction and in magnitude of the relationship with panel score for the 3 types of meat. Thus, it is evident that irradiation flavor is not consistently associated with any of these components. Examination of the differences between the species involved indicates that the variation in response to radiation may be related to the level of these components, or perhaps more likely to causative effects of variation in these components.

Amount of glycogen, pH and level of total glutathione did not appear to influence panel score consistently in either direction or magnitude. However, glycogen disappeared on cooking and could be determined in only four samples, so it is possible that the amount present in irradiated raw meat may exert an influence on acceptability. Similarly, the effect of total glutathione could not be determined, since values were not obtained for the beef, where a significant relationship existed between reduced glutathione and panel score ($r = .48$).

Multilinear Correlation Coefficients

Table 3 contains a summary of the multilinear correlation coefficients for various combinations of hydrogen sulfide, methyl mercaptan and carbonyls with mean panel scores. Examination of the relationships for beef reveals that hydrogen sulfide and methyl mercaptan combined ($R = .78$) showed very little improvement over methyl mercaptan alone ($r = -.76$).

TABLE 3

Multilinear correlation coefficients of various chemical components in irradiated meat with panel scores

	Beef	Pork	Veal
H_2S-methyl mercaptan	.78	.55	.50
H_2S-carbonyls	.88	.58	.59
Methyl mercaptan-carbonyls	.87	.65	.49
H_2S-methyl mercaptan-carbonyls	.93	.65	.61

When hydrogen sulfide ($r = -.46$) was combined with carbonyls ($r = -.74$), the multilinear relationship showed considerable improvement over either single correlation coefficient, with an "R" value of .88. Thus, results suggest that carbonyls and hydrogen sulfide may combine to produce some of the off-flavors present in irradiated beef. Similarly, methyl mercaptan and carbonyls, when combined to give multilinear correlation coefficients, show considerable improvement over either single correlation coefficient (Tables 2 and 3). When hydrogen sulfide, methyl mercaptan and carbonyl values were combined to give a multilinear correlation coefficient with taste panel score, there was a further improvement in the relationship over any two-way combination with a value of .93. Thus, a combination of hydrogen sulfide, methyl mercaptan and carbonyls accounted for approximately 86% of the variation in panel score.

With pork, there was little or no added advantage to combining hydrogen sulfide with either methyl mercaptan or carbonyls, but there was measurable

improvement in the relationship when methyl mercaptan and carbonyls were combined (Tables 2 and 3). However, the multilinear relationship of hydrogen sulfide, methyl mercaptan and carbonyls resulted in no improvement over that of methyl mercaptan and carbonyls combined.

With veal, hydrogen sulfide and methyl mercaptan combined (Table 3) resulted in no increase in the relationship over the simple correlation of hydrogen sulfide and panel score (Table 2). Hydrogen sulfide and carbonyls combined resulted in some improvement over the simple relationships with panel score (Tables 2 and 3), but the combination of methyl mercaptan and carbonyls showed no improvement over the simple correlation coefficients. Similarly, there was only slight improvement in the multilinear relationship when hydrogen sulfide, methyl mercaptan and carbonyls were correlated with panel score over the two-way combination of hydrogen sulfide and carbonyls.

Results of the study indicate that the carbonyls appear to either enhance the undesirable flavor effects of methyl mercaptan and hydrogen sulfide or else combine to produce the objectionable flavor of irradiated beef. If the flavor effect is due to a combination of the 3 compounds, it could either be a simple mixture of the right proportion or perhaps formation of a different compound, which is responsible for the flavor problem. The fact that carbonyls combined with either methyl mercaptan or hydrogen sulfide appeared to account for a portion of the off-flavor, above and beyond that of any one of these single compounds; all three combined together accounted for a greater percentage of the variability in score, would favor the theory of a simple mixture.

Results of the pork and veal investigations are not as clear-cut. This is not surprising, when one considers that the flavor of pork and veal is not so adversely affected by irradiation as is true for beef *(10)*. However, it is noteworthy that the multilinear relationship of hydrogen sulfide and methyl mercaptan with panel score was not improved, which was in agreement with the results for beef. In contrast to beef, neither the combination of hydrogen sulfide and carbonyls nor the three-way combination for pork resulted in any further improvement in the relationship to panel score. Whereas, for veal the only multilinear relationship which is improved measurably over the simple relationship is that of hydrogen sulfide and carbonyls. Thus, carbonyls again seem to be associated with irradiation flavor, although the effect is not so evident.

In interpretation of the simple and multiple correlations reported in this study, it should be pointed out that the off-flavor of irradiated meats is due to irradiation *per se*. Thus, if level of radiation were the only variable being studied, there would be an automatic correlation between panel score and any chemical component that increased or decreased directly or inversely with score. However, in this study not only level of radiation was varied but also the length of storage at various temperatures. Therefore, it is unlikely that any chemical component other than those responsible for the off-flavors would vary with panel scores. Analysis of variance *(14)* was applied to the data to test the effects of radiation level upon acceptability ratings. Results reveal that the differences between the two higher levels of radiation were not significant. Although, the unirradiated beef and veal were rated significantly higher than the irradiated, with pork there was no dif-

ference. In final analysis, if the level of certain chemical components can be used to predict panel scores, it matters little whether changes in the chemical components are directly responsible for panel scores or if the relationship is strictly automatic.

SUMMARY

The relationship between certain chemical components and the panel score of irradiated meats was investigated. Simple and multilinear correlation coefficients indicated that hydrogen sulfide, methyl mercaptan and carbonyls were responsible for a considerable part of the poor acceptability of irradiated beef, pork and veal. The combined effects of hydrogen sulfide, methyl mercaptan and carbonyls accounted for 86, 42 and 37% of the variability in panel scores for irradiated beef, pork and veal, respectively.

Acknowledgment

The authors are indebted to Mrs. Eldora Spooner and Mrs. Sally Hruska for preparing the meat and conducting the taste panels and to Dr. W. T. Magee for advice in the statistical analysis. The authors also gratefully acknowledge the assistance of Mr. J. V. Nehemias from the Fission Products Laboratory, University of Michigan, Ann Arbor, Michigan, for supervising the radiation of the samples, and the counsel of Dr. B. S. Schweigert and Dr. D. M. Doty of the American Meat Institute Foundation in carrying out the experiment.

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FEEDING TEST WITH CARRAGEENIN

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Carrageenin is the hot-water-soluble extractive of the red seaplants *Chondrus crispus* and *Gigartina mamillosa*, and other related species commonly referred to as Irish moss *(5)*. The commercial product contains about 12% moisture and about 80% of the polysaccharide hydrocolloid, which is precipitated in 70% isopropanol. The remaining soluble materials are metallic chlorides and sulfates, and low-molecular-weight organic compounds. About 23% by weight of the hydrocolloid is ash and no fillers are used to standardize the physical characteristics of the commercial product.

The hydrocolloid is now known to be composed of two distinct types of galactosan sulfates (kappa and lambda) in varying but usually equal proportions plus minor quantities of floridean starch, which is a glucosan resembling glycogen. Both kappa and lambda carrageenins consist of sulfated D-galactose units, which apparently must exist in a distinct and definite structural relationship with one another and cannot occur in large separate aggregates *(1, 6)*.

A carrageenin sol is a strongly charged polyelectrolyte with a reported ionization of the sulfates of approximately 60% *(2)*. There is one sulfate unit for almost every hexose unit. Sols of carrageenin are very viscous at low concentration and form thermally reversible gels on addition of specific cations, particularly potassium, ammonium, and calcium.

Because of its valuable colloidal properties and availability in commercial quantities as a refined product of controlled quality, carrageenin has been used extensively in frozen confections; dairy products such as ice cream, chocolate milk and processed cheeses; puddings; bakery and fountain supply specialties; oil emulsions; toothpastes; hand lotions; and various other food, cosmetic, and pharmaceutical preparations. In all of these applications the concentration of carrageenin is less than 1% by weight of the product.

The feeding studies reported here were conducted under a cooperative arrangement with the Seaplant Chemical Company, New Bedford, Massachusetts, in order that the U. S. Bureau of Commercial Fisheries might obtain data on the nutritive value of sea plant products so as to be better able to recommend rational use of this important natural resource.

EXPERIMENTAL

The product used in the feeding studies from 1951 to 1953 was Krim-Ko-gel, High Viscosity (now designated Sea Kem, type 5), which was purchased on the open market except for several lots which were supplied by the manufacturer at the request of the Bureau. The first 2 lots analyzed respectively, 76 and 75% hydrocolloid, 12 and 15% moisture; 25 and 22% mineral matter; by weight. The hydrocolloid content was based on precipitating a 2% solution of the sample with 2 volumes of 99% isopropanol and was the best available index of gum content. The range in viscosity for the 7 lots of carrageenin

used was 180° to 210° MacMichael (2% solution at 40° C., large bob at 20 r.p.m. and no. 26 wire). The gel strength varied from 30 to 60 g. (force in grams transmitted through a 1.1 cm. diameter plunger to rupture a 2% gel of sample aged for 1 hr. in a 25° C. water bath). The arsenic content varied from 0.04 to 10.4 parts per million (Gutzeit test). Since the arsenic content was quite high, a concerted effort was made to look for evidence of toxicity during the conduct of these tests and during the microscopic examination of tissue samples of organs after death of the animals. No histologic evidence of toxicity due to arsenic ingestion was found.

Male and female mice and rats of 2 strains each were allotted at weaning time to groups of 10 animals each. They were housed individually in screen metal cages on wire-mesh floors. The animal room was maintained at 80° F.

The diet used was "Feed, Dry, for Laboratory Animals" (proposed Federal Specification, May 24, 1949, General Services Administration, Washington 25, D. C.) made up of natural feed ingredients. The protein was furnished by dried skim milk, fish meal, animal liver meal, meat scrap, soybean oil meal, wheat, barley, oat groats, corn and alfalfa meal. Supplementary vitamin sources were fish liver oil, wheat germ meal, dried brewer's yeast and dried whey fermentation solubles. This diet contained not less than 23% crude protein and 5% crude fat, and not more than 4% crude fiber and 7% mineral matter. The carrageenin was mixed into the diet at 1, 5 and 15% levels at the expense of equal weights of ground yellow corn, and at the 25% level for a fixed ratio of ground yellow corn, wheat, and oats. These are manifestly high levels of carrageenin, since it is hardly ever included at levels as high as 1% on a dry-matter basis in commercial products. The rats were weighed once a week at which time also the food and water intake for the week was determined. No intermediate data were gotten for the mice. All rats and mice were kept until death (Table 1) at which time a necropsy was done to determine the presence of

TABLE 1

Survival rate of rats and mice fed different levels of carrageenin

Diet designation		Number on test	Survivors after months of test							
			3	6	9	12	15	18	21	24
Rats:										
Control										
(1951)		10	10	9	9	8	7	7	6	3
Carrageenin:										
(1951)	1%	10	10	9	9	9	7	6	4	0
"	5%	10	10	9	8	6	5	4	3	3
"	15%	10	10	10	10	10	9	9	5	2
"	25%	10	10	10	9	8	8	7	3	2
(1939)	5%	10 [1]	10	9	9	9	8	6	5	2
Mice:										
Control										
(1951)		10	9 [2]	9	9	7	5	4	1	0
Carrageenin:										
(1951)	1%	10	10	10	9	7	6	1	1	0
"	5%	10	9	9	7	7	6	4	1	1
"	15%	10	8 [3]	6	5	5	5	4	2	1
"	25%	10	10	10	8	8	7	6	5	4

[1] Half of the rats had received the 10% level in the diet for the first 10 weeks.
[2] Mouse escaped from cage after 11 weeks.
[3] Mouse killed accidentally after 6 weeks.

gross evidences of disease. Organ sections or entire organs of each animal not too badly decomposed were preserved in buffered formaldehyde solution. Later, these were sectioned and examined microscopically, and an evaluation was made of the probable reasons for death.

For comparison, previously unreported data are included for lifetime studies of male rats of the Yokelson strain fed the 5% level of Irish moss gum for which the data for the first 10 weeks have been reported by Nilson and Schaller *(3)*. The diet fed in this instance

consisted of Irish moss gum, lactalbumin, sucrose, dextrin, lard, brewer's yeast, wheat germ, cod liver oil, and salt mixture. The control diet contained about 20% crude protein, 18% crude fat, 48% carbohydrates and 5% mineral matter. Data for the control rats of this series have been reported by Nilson and Wagner *(4)*.

RESULTS AND DISCUSSION

Data in Table 1 indicate that the animals consuming diets containing the different levels of carrageenin lived as long as those eating the control diet without sea plant gum. Female rats and mice generally lived longer than did males, but there is no evidence that diet was a factor. It is concluded that the diets consumed were apparently not a factor in determining length of life.

Only the rats consuming the diet containing the 25% level had lesions suggesting hepatic cirrhosis, which could indicate some dietary deficiency or insufficiency. The incidence was not particularly high, and so the deficiency related to the above was probably marginal. Respiratory infections were the chief cause of death of the rats in all groups. There were lesions of many origins present, but no single type of lesion was consistent in the rats of all groups. Rats consuming diets containing less than the 25% level of carrageenin showed no evidence of injury to parenchymatous organs directly or indirectly. These conclusions, based on gross observations on necropsy, also applied to the rats of the 1939 series.

There was no evidence of damage due to nutritional deficiency in the mice. The studies indicated that respiratory infection, suppuration, and metastatic abscesses were frequently responsible for death. Malignancy was fairly common in all groups, including mammary carcinoma, leukemia, and lung tumors.

In summary, most of the rats and mice suffered from extensive pulmonary infections with metastasis. There were no evidences of lesions of nutritional origin except for the cirrhosis found in the group of rats eating the diet containing 25% carrageenin. Except possibly in this instance, there was no evidence of toxic, corrosive, or other deleterious effects of the diets on the gastro-intestinal tract, kidneys, or livers. The data for these species indicate that carrageenin is a wholesome product except possibly at levels in excess of about 20 times the highest level used in foods. Also, these foods normally supply only a small fraction of the total food intake of the consuming public.

A study of utilization of carrageenin by the rat would be useful, since the data on length of life and reasons for death did not indicate that the diets were implicated to any significant extent. Data in Table 2 show that only with rats consuming the diet containing the highest level fed (25% carrageenin) was the mean maximum live weight reduced. These rats averaged about 90% of the mean maximum weight of the negative control group, and their mean weight was barely statistically significantly different at the 5% level from the mean maximum weight of the rats fed the 5% level of carrageenin, namely, 252 and 296 g. respectively. This decrease in size was apparently due to lowered intake of essential nutrients. There was no indication that the other differences in mean maximum weight were due to unwholesomeness of any diet ingredients, but was determined by the different numbers of the 2 sexes in the groups, the earlier mortalities due to non-specific reasons and strain of rats.

TABLE 2

Data on feeding tests with carrageenin conducted from weaning to death of the rats

	Diet designation					
	Control	Level of carrageenin, %				
		1	5	15	25	5
	(1951)	(1951)	(1951)	(1951)	(1951)	(1939)
Number of rats	10	10	10	10	10	10
Mean maximum weight, g.	289	334	296	305	252	610 [1]
Coefficient of variation, %	30	34	23	25	20	25
Mean weekly basal diet intake, g.	90	101	103	117	118	98
Coefficient of variation, %	20	26	17	18	11	17
Mean weekly water intake, g.	222	250	265	313	348	197
Coefficient of variation, %	10	24	18	21	16	18
Mean apparent digestibility of carrageenin during tenth week, %		64	51	41	46	52
Coefficient of variation, %		39	35	24	28	29

[1] Male rats only of the large growing Yokelson strain.

Data for mean food intake indicate that the rats consumed more food, from 101 to 118 g. weekly, as the level of carrageenin increased, in order to try to ingest maximum nutrients. Mean weekly intakes of carrageenin during the 1951 experiment were 1, 5, 18, and 30 g., respectively. The increased mean weekly water intake was 28, 8, 5, and 4 g. per g. of carrageenin ingested as the level in the diet increased from 1 to 25%. This decrease of water intake per gram of carrageenin was probably determined by the physical limit in the volume of food that could be handled by the gastro-intestinal tract.

Studies indicated that the mean apparent digestibility by rats of carrageenin during the tenth week of the experiment was roughly 50% irrespective of the level fed. It cannot be determined from these studies how much was actually used by the animals and how much was used by bacteria. The finding that the rats fed the diet containing the 25% level of carrageenin may have suffered nutritional deficiency or insufficiency is not surprising, since the bulk handled was large and the feces were often very loose, suggesting that mechanical loss of nutrients could not be discounted. The surprising finding is that these animals, as well as those receiving the 15% level, could handle such volumes of hydrated gums with so little apparent discomfort. Only one rat, at necropsy showed any evidence of impaction of intestines, and it consumed the diet containing the 15% level of carrageenin. In commercial practice the carrageenin is hydrated and used in such small quantities that a high consumption rate by humans is not a problem.

The mice were fed free-choice the same diets and water as fed to the rats. Food or water intake data were not kept. In general, all mice seemed to be able to consume enough nutrients to maintain well-being and there was no trouble with impaction, dermatitis, or excessively soft feces.

CONCLUSIONS

The data indicate that carrageenin is free from any deleterious substances which adversely affect the health of rats and mice or utilization of nutrients, except possibly for mechanical interference when excessively high levels are fed.

Acknowledgment

The authors wish to thank the staff of the Research Division of Seaplant Chemical Company for the chemical analysis of coded samples of carrageenin used, and the determination of carrageenin content of samples of feces collected in order to determine apparent digestibility.

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THE ODOR OF METHIONAL PURIFIED BY GAS CHROMATOGRAPHY

Methional has been cited by Patton and others *(1, 2, 3)* as an odoriferous component in a variety of processed foods. On the other hand Witting and Batzer *(5)* have stated recently that freshly vacuum distilled methional is odorless and that the aldehyde develops a distinct odor after a short exposure to air, presumably, as the result of air oxidation. More recently Patton and Barnes *(4)* have reported that as judged by several observers vacuum distilled methional is overwhelmingly odorous and that the odor intensity does not increase by exposure to air.

The application of gas chromatographic techniques to the purification of methional offers an opportunity to decide on the divergent claims made on the odor of this compound.

Methional was prepared by the method of Patton and Barnes *(4)*. The crude reaction product was purified by distillation at 40.5 mm. Hg pressure and the main fraction which distilled from 85 to 86° C. was taken as the methional. This fraction had a strong distinct odor as evaluated by several observers.

Analytical gas chromatography of the product was carried out in a Perkin-Elmer fractometer. Column packings of dinonyl phthalate, Carbowax 500 and DC-silicone fluid 200 (Perkin-Elmer "C") were used and yielded in each case only one major single component. The Perkin-Elmer column "C" was selected as it gave the most symmetrical peak for methional. The results and the experimental conditions are given in Figure 1. A main peak corresponding to methional and amounting to more than 99% of the chromatographed sample was recorded. Two highly volatile impurities amounting to

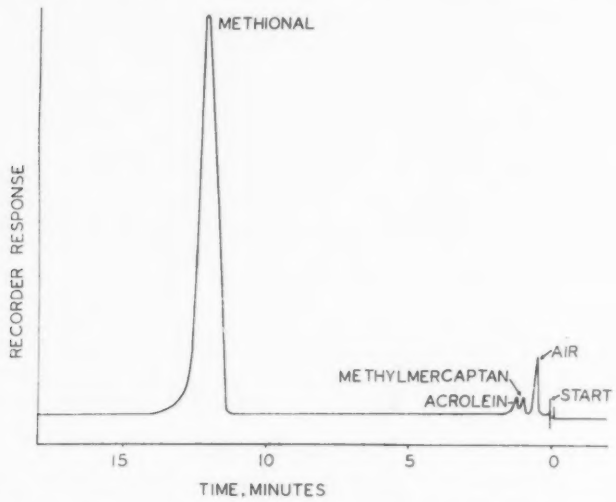

Figure 1. Chromatogram of distilled methional. Perkin-Elmer "C" column, 85° C. 25 p.s.i. He

much less than 1% were identified as methyl mercaptan and acrolein. The odor of methional was tested as it emerged from the column and it was found to be essentially the same as that of the sample before gas chromatography. Perhaps, the only difference was that no traces of methyl mercaptan odor could be detected.

The isolation of the pure product in measurable amounts was subsequently carried out using a Perkin-Elmer preparative-scale column under similar conditions to those previously described. Samples of 0.2 and 0.5 ml. were injected into the chromatograph and the methional was collected in a U-tube immersed in liquid nitrogen.

From these chromatographed samples, the infrared spectrum and the dinitrophenylhydrazone were obtained. The infrared spectrum was in agreement with that published by Patton and Barnes *(4)* and the dinitrophenylhydrazone melted at 123-4° C., (corrected 122-3° C.). All the samples obtained were found to have, without exception, the same characteristic odor.

The relatively low temperature of the chromatographic separation, the inertness of the carrier gas used and the low temperature at which the aldehyde was collected make it unlikely for an oxidation to take place. The conclusion is that methional itself is an intensely odorous compound.

It is then possible that methional, perhaps in combination with methyl mercaptan and other odorous compounds, may be responsible for the odor of methionine-containing proteins that have been irradiated by gamma rays.

Work is in progress in order to establish a comparison of the odors of methional, its sulfoxide and sulfone by means of a new reactor-gas chromatographic technique *(6)*.

The authors acknowledge the technical assistance of Mrs. Jean Allison for the infrared spectrum.

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The Editor, *Food Research*

Dear Sir:

Additional comments concerning the use of the gallon-size Waring blender noted by Hartman, Paul A. in "A Composite Sample Method for the Analysis of Blended Materials" (*Food Research*, **23**, 565, 1958) may be of interest to readers of this journal.

We have used these gallon-size stainless steel blenders for over a year to obtain homogenates of whole, raw, and cooked crabs. In several studies dealing with the bacteriology of crabs and crabmeat, it was desired to sample whole crabs rather than only picked meat of the musculature as is commonly practiced. Accordingly, we remove carapace, chelae and swimming legs and place the remaining 'core' into the sterile, chilled blender. To this we add sufficient sterile chilled distilled water to achieve a desired dilution. We have found that blending for 30 seconds to one minute at low speed and two minutes at high speed achieves an homogenate free of unpipettable particles. In this way, 4 or 5 crabs can be sampled simultaneously, depending upon their total weight.

After use, the container is washed, dried, and aluminum foil placed over its mouth. Following autoclaving and cooling the container is placed in a refrigerator (1-3° C.) until needed. In order to avoid damage to the vinyl gasket of the closure lid, it is not autoclaved. The lid is wrapped in aluminum foil and generously flamed off just before use. Thus, we are able to use one lid continuously.

In addition to sampling whole crabs and shucked oysters, we have found this blender quite satisfactory for the preparation of seafood spreads and pastes and for mixing ingredients for crab cakes.

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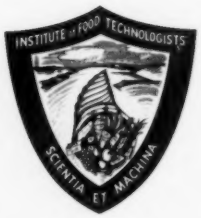
November 20, 1958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